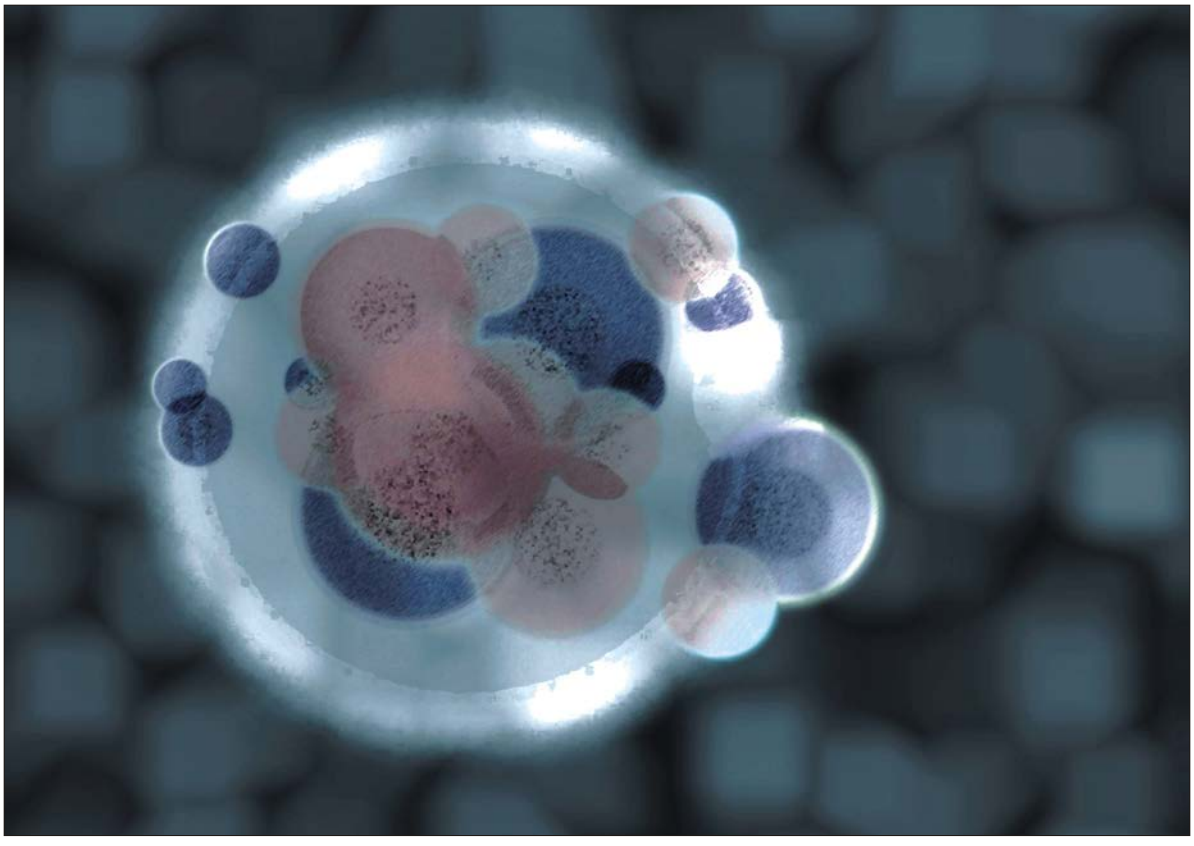

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Removal of Arsenic Using *Acetobacter xylinum* Cellulose

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Abstract: The removal of Arsenic from solutions using adsorption in *Acetobacter xylinum* cellulose is described. The adsorption was determined, along with the effect of different variables, such as adsorbent dose, reaction time, arsenic concentration and pH, on the efficiency of arsenic removal. It was concluded that the adsorption is fast and improved under conditions of alkalinity. The kinetic experiments showed that the process equilibrium was reached quickly as a function of pH and less than 10 μg L^{-1} was achieved from an initial concentration of 100 μg L^{-1}. It was found that the equilibrium dependence between adsorption capacity and bulk metal ion concentration could be described with the isotherm. In the isotherm studies, the observed data fitted well with both the Freundlich and the Langmuir models. The adsorption mechanism is a result of complex formation between arsenic and the microbial cellulose. A selective desorption can be carried out using a 1 mol L^{-1} phosphoric acid solution. The microbial cellulose was found to be a very efficient adsorbent.

Key words: Adsorption, arsenic, *Acetobacter xylinum*, cellulose, desorption

INTRODUCTION

Contamination of the environment with arsenical (As) from both natural and anthropogenic sources is widespread, occurs in many parts of the world and is, therefore regarded as a global issue (Smedley and Kinniburgh, 2002). Arsenic exists in the -3, 0, +3 and +5 oxidation states. Environmental forms include arsenious acids (H_3AsO_3, H_3AsO_3, $H_3AsO_3^{2-}$), arsenic acids (H_3AsO_4, H_3AsO^{4-}, $H_3AsO_4^{2-}$), arsenites, arsenates, methylarsenic acid, dimethylarsinic acid, arsine, etc. (Smedley *et al*., 2002). Generally, As(V) is more prevalent in surface water while As(III) is more likely to occur in anaerobic ground waters (Vaishya and Gupta, 2005). However, actual valence states depend on the redox environment in water systems and may vary from districts to districts. Arsenicals are introduced in the aqueous system through geochemical reactions, industrial wastewater discharges, or agricultural use of arsenical pesticides. The toxic and carcinogenic effects of As on living organisms are well documented (Murugesan *et al*., 2006). In most countries, the As level of water is limited to 0.05 mg L^{-1} (Smedley and Kinniburgh, 2002). Therefore, a treatment process is necessary to remove As from industrial wastes in order to reduce its concentration. The World Health Organization (WHO) provisional guideline of 0.01 mg L^{-1} has been adopted as the drinking water standard (WHO, 1996). However, many countries have retained the earlier WHO guideline of 0.05 mg L^{-1} as their standard or as an interim target. The US Environmental Protection Agency (USEPA) recently revised the maximum contaminant level for As in drinking from 0.05 to 0.1 mg L^{-1}. The maximum contaminant level of 0.05 mg L^{-1} had been the standard since 1942 (USEPA, 2001). Independent review of toxicity data by the National Research Council regarding human health risk from As exposure concluded that chronic ingestion of inorganic As causes bladder, lung and skin cancers. Removal of As is one of the most important areas of water and wastewater treatment (Pagnanelli *et al*., 2000). The demand for effective and inexpensive methods to and/or remove As from water sources is in response to the widespread recognition of the deleterious health effects through this source. Usually, the requirements for a removal technique of As from aqueous system are: (1) safe operation with respect to the maximum contaminant level, (2) high efficiency, (3) easy for application and (4) low cost (Loukidou *et al*., 2003). Various treatment methods such as ion exchange, adsorption, ultra filtration, reverse osmosis and adsorption coprecipitation by metals (predominately ferric chloride) followed by coagulation

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have been so far proposed and adopted for the removal of As from aqueous media (Sharma *et al*., 2006). Adsorption technique is generally considered to be a useful method and has been studied for the removal of toxic metals from dilute aqueous solution. The adsorption is a mass transfer process where a substance is transferred from the liquid phase to the surface of a solid and becomes bound by chemical or physical forces. Selective adsorption utilizing biological materials, mineral oxides, activated carbons, or polymer resins, has generated increasing interest (Davis *et al*., 2003). However, for most current methods and media, there is a problem in terms of either efficiency or cost. Therefore, new techniques are required that can effectively reduce As concentration to environmentally acceptable levels at affordable costs. The objective of this study was to examine a new adsorbent for the removal of As, employing *Acetobacter xylinum* cellulose.

MATERIALS AND METHODS

The microbial cellulose production: *Acetobacter. xylinum* was isolated in Bacteriology Department of Tarbiat Modares University. It was grown in SH medium at 28°C under static culture condition. SH medium was composed of 2% (W/V) glucose, 0.5% (W/V) Yeast extract, 0.5% peptone, 0.27% (W/V) Na_2HPO_4 and 0.115% (W/V) citric acid (Hestrin and Schramm, 1954). The cellulose sheets after cultivation were removed and rinsed with distilled water and devoided from bacterial and medium residues using 2% sodium dodecyl sulfate and 4% NaOH solutions in a bath of water at boiled temperature. The microbial cellulose was dried at 70°C.

Batch adsorption experiments: Batch adsorption experiments were performed in conical flasks stirred in a shaker at 100 rpm at ambient temperature 25°C and for different pH values. An aqueous solution of As of known concentration (prepared from $Na_2HAsO_4.7H_2O$ salt) was added to the microbial cellulose in suspension. The solution pH was adjusted by the addition of HCl or NaOH solutions. The residual concentration of As was analyzed after the separation of used adsorbent. The solution was allowed to settle for 1 h, filtered and analyzed for As. The As content of the solution before and after adsorption represented the amount of As adsorbed by the adsorbent. The samples were analyzed for As using Perkin Elmer atomic absorption spectrophotometer with graphite furnace accessories (Limit of Detection of the instrument is 2 μg L^{-1}). Argon gas of ultrahigh purity (99.995%) was used to sheath the atomiser and to purge it internally. An As hallow cathode lamp (Varian Canada Inc., Toronto) was used with emitting wavelength of 193 nm with a slit width of 0.5 nm. An external reference standard was used to verify the calibration. Prior to analysis, the aqueous samples were acidified with concentrated nitric acid in an amount of 1% and stored in acid washed high density polyethylene containers (Harvey, 2000).

Adsorption isotherms: The adsorption data were fitted to Freundlich and Langmuir isotherm models. The linearized form of the Freundlich equation is:

$$\log q_e = \log K_{f+} [(1/n) (\log C_e)]$$

Where:

C_e = The As concentration in solution at equilibrium,
q_e = The mass of the microbial cellulose per unit weight at concentration,
C_e, k_f and n = Constants.

The linearized Langmuir equation given as:

$$C_e/q_e = (1/ab) + (C_e/a)$$

Where:

q_e and C_e = The same as for the Freundlich equation,
a and b = Constants (Altundogan *et al*., 2002).

Arsenic desorption: Several elutants were investigated, such as hydrochloric acid, nitric acid and phosphoric acid, by putting a fixed amount of the microbial cellulose of known As concentration in contact with a fixed volume of elutant at a controlled pH. For desorption study, initial adsorption was carried out with 0.4 g of the adsorbent and 100 mL of 0.1 mg L^{-1} As at the pH of mother solution using the procedure adopted so far. Thereafter, the pH of the solution was adjusted to the desired pH by controlled addition of dilute HCl or dilute NaOH. The solution was shaken further for half an hour in a wrist shaker and allowed to settle for another hour before it was filtered and analyzed for As.

RESULTS AND DISCUSSION

Acetobacter xylinum cellulose: The thick, gelatinous membrane formed in static culture conditions. The cellulose produced in the form of a gelatinous membrane can be molded into any shape and size during its synthesis, depending on the cultivation technique and conditions used. *A. xylinum* is an important industrial microorganisms used in the production of bacterial cellulose (Geyer *et al*., 1994).

Effect of solution pH: The evolution of pH during the adsorption experiments, shown in Fig. 1. The pH of the solution influences both the metal ionic forms and the ligands responsible for binding of metal ions at the cell surface (Rao *et al*., 1993). Thus, the adsorption is strongly influenced by the pH of the solutions (Mameri *et al*., 1999). These result demonstrates that low pH limits the adsorption of As by the microbial cellulose indicating that the ion exchange between As and hydronium ions of the cell surface is involved to some extent in the sorption mechanism. At low pH, the cell wall is protonated, inducing a weak complexation affinity between the microbial cellulose and the As. Further more, As is highly soluble in an acidic medium decreasing adsorption efficiency. However, as pH increases, As solubility decreases promoting adsorption, electrostatic repulsion between metal and cell wall decreases favor complexing. In addition, the generation of hydrolyzed species is followed by an increase of ionic size which facilitates contact between functional sites and metal (Guibal *et al*., 1992). This increase in adsorptive capacity continues until metal precipitates (Volesky and Holan, 1995). In an attempt to determine the extent of ion exchange, a comparison of the quantities of the As adsorbed and the protons released revealed that there was a linear increase in the quantity of released protons as the quantity of As removed increases. These results implied that, ion exchange in between As and protons of the cell surface is not the only mechanism, but that some other mechanisms may be involved leading to the release of protons into solution as sorption proceeds.

Effect of adsorbent dose on As removal: The effect of adsorbent dose on the adsorption of As has been studied at pH 7.0 with adsorbent dose varying from 100 to 1000 mg L^{-1} and at a fixed initial As concentration of 1 mg L^{-1}. It is apparent that the removal efficiency, defined as the ratio of equilibrium and initial concentration of As increased with adsorbent dose from a given initial solute concentration (Fig. 2). At 400 mg L^{-1} of adsorbent, when adsorbent dose increased from 100 to 1000 mg L^{-1}, the removal efficiency reached approximately 90%. The most important factor was how many adsorption sites remain unsaturated during the adsorption process. This is due to the fact that as the dose of adsorbent increased, there is less commensurate increase in adsorption resulting from the lower adsorptive capacity utilization of the adsorbent (Honeyman and Santschi, 1988).

Batch isotherm studies: The data obtained from the isotherm studies was used to analyze the adsorption isotherms in order to estimate the constants, adsorption density and adsorption maxima. Several isotherm equations have been used for the equilibrium modeling of adsorption systems (Altundogan *et al*., 2002). In this

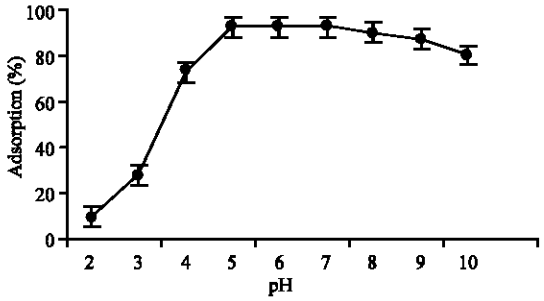

Fig. 1: Effect of pH on percentage of adsorption (temperature: 25°C, agitation rate: 100 rpm, agitation time: 30 min, adsorption dose: 100 mg L^{-1}, As concentration: 1 mg L^{-1})

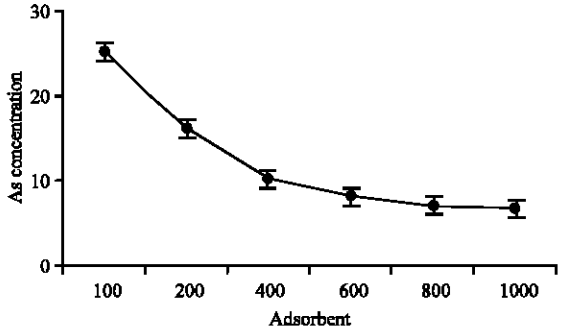

Fig. 2: Effect of adsorbent on As removal (temperature: 25°C, agitation rate: 100 rpm, agitation time: 30 min, adsorption dose: 100-1000 mg L^{-1}. As concentration: 0.0-30 μg L^{-1})

study, Langmuir and Freundlich isotherms were applied. For each isotherm the initial As concentrations were varied between 1 and 10 mg L^{-1}, whereas the microbial cellulose concentration was kept constant at 1 g L^{-1}. An adsorption isotherm is characterized by certain constants, values that represent the surface properties and the affinity of the adsorbent. They can also be used to evaluate the adsorptive capacity of the microbial cellulose. High regression correlation coefficients were found (>0.97) for the microbial cellulose, suggesting that both models are suitable for describing the adsorption equilibrium of As by the microbial cellulose. This observation implies that monolayer adsorption, as well as heterogenous surface conditions may coexist under the applied experimental conditions (Sag and Kutsal, 1995). Hence, the overall adsorption of As on the biomass is complex, involving more than one mechanism, such as ion exchange, surface complexation and electrostatical attraction. The magnitude of K_f and n values showed the easy adsorption of As and the high adsorptive capacity of the microbial cellulose. The constant K_f is an indication

of the adsorption capacity of the adsorbent, while the parameter n indicates the effect of concentration on the adsorption capacity and represents the adsorption intensity. The value of n; which is related to the distribution of bonded ions on the adsorbent surface, was found to be greater than unity for all the modified biomasses, indicating that adsorption of As is favorable (Sag and Kutsal, 1995). Nevertheless, it has to be noted that the Freundlich model does not describe the saturation behavior of the adsorbent Q_o; whereas the Langmuir constant represents the monolayer saturation at equilibrium. This means that Q_o is the maximum value of q_{eq} that is important to identify which adsorbent show the highest adsorption capacity. As such it is useful for scale up considerations. A high value of (b) also implies strong bonding of As to the microbial mediuni at ambient temperature. The highest value of b was found to be 0.08 for the adsorbent (Tarasevich and Aksenenko, 2004).

CONCLUSIONS

Based on the present study, it was conclude that microbial cellulose is effective in reducing As. However, the kinetic study indicates that less than 10 μg L^{-1} of As could be achieved at the pH levels of 7.0 achieving equilibrium at 2 h. According to the isotherm studies, both Freundlich and Langmuir models were applicable and statistically significant. This initial study demonstrated promising use of natural matrices, *A. xylinum* cellulose, for As removal, however, optimal conditions and the definition of temperature, dissolved organic constituents, redox condition and the presence of microorganisms in water and wastewater need to be defined.

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