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Adsorption of Mercury from Synthetic Solutions by an *Acetobacter xylinum* Biofilm

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Abstract: The aim of this research is to study the adsorption of mercury from aqueous media by an *Acetobacter xylinum* biofilm. Effects of pH, adsorption time, initial concentration and the adsorbent dosages on the adsorption of mercury were studied. The influence of pH on the adsorption onto the biofilm was studied in the pH range. The surface charge density varied with pH and the concentration of mercury adsorbed significantly increased from pH 5.0 to maximum levels at pH 8.0. Adsorption equilibrium was established after about 50 min, after which point the level of adsorbed mercury did not significantly change with time. The adsorption equilibrium was also represented with Langmuir and Freundlich adsorption isotherms. The adsorption capacity was 180 mg g^{-1} . Desorption studies were performed with hydrochloric acid. The results suggest that an *Acetobacter xylinum* biofilm could be used as an effective adsorbent for the removal of mercury.

Key words: Adsorption, mercury, cellulose, *Acetobacter xylinum*, biofilm

INTRODUCTION

Mercury is generally considered to be one of the most toxic metals found in the environment. This heavy metal may be introduced into the natural environment in any form and from a variety of sources, at which point it can be converted into more toxic volatile forms (i.e., methyl mercury chloride, ethyl mercury chloride and phenyl mercury chloride) by microorganisms and abiotic processes (Yardim *et al.*, 2003; Choong and Park, 2005). In an aqueous environment, mercury in sediment is subject to methylation, forming the more toxic methylmercury. Once mercury enters the food chain, larger accumulation of mercury compounds takes place in humans and animals (Heyes *et al.*, 2004). The major sources of mercury pollution in the aquatic environment are industries such as chloralkali, paint, pulp and research, oil refining, electrical, rubber processing and fertilizer (Manohar *et al.*, 2002). Mercury causes damage of the central nervous system and chromosomes, impairment of pulmonary function and kidneys, chest pain and dyspnoea. Hence it is essential to remove mercury from wastewaters before its transport and cycling into the environment (Kacar *et al.*, 2002). Various types of technology are available for removing mercury from water and wastewater. These include chemical precipitation, conventional coagulation, lime softening, reverse osmosis, ion-exchange and activated carbon adsorption (Cyr *et al.*, 2002). The use of biological materials for heavy metal removal and recovery technologies has gained high credibility during recent years, due to high-quality performance and low cost of the materials. Developments in the field of environmental biotechnology indicate that bacteria, fungi, yeast and algae and their products can remove heavy metals from aqueous solutions by adsorption (Roux and Fourest, 1997). Cellulose is the most common biopolymer in the world, with an estimated production of 10^{11} ton/year. Most of this is produced in plant cell walls. The most

popular and industrially important isolation of cellulose from plants including separation processes to remove lignin and hemicelluloses. The other way consists of the biosynthesis of cellulose by different types of microorganisms. Several bacteria are in condition to produce cellulose as reported from strains from the genera *Acetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium* and *Sarcina*. *Acetobacter xylinum* is a gram-negative aerobic bacterium that secretes cellulose fibers as part of its normal metabolic activity (Rainer and Luiz, 1998). The cellulose synthesized by *Acetobacter xylinum* is identical to that made by plants with respect to its molecular structure. However, the secreted polysaccharide is free of lignin, pectin and hemicellulose, as well as biogenic products, which are associated with plant cellulose (Hong *et al.*, 2001). This cellulose has high crystallinity, high water absorption capacity and mechanical strength in the wet state, ultra-fine network structure and availability in an initial wet state (Astley *et al.*, 2001). Because of these features there is an increasing interest in the development of new fields of application (Klemm *et al.*, 2001). A wide variety of natural products, composed primarily of cellulosic matrix, were investigated by different researchers for their ability to remove various heavy metals from aqueous and nonaqueous solution (Bailey *et al.*, 1999; Chakravarty *et al.*, 2007). These included pine bark, peanut shell, saw dust, cotton, shea butter seed husk and sunflower stalk, among others. A comprehensive list of naturally occurring adsorbents for the removal of heavy metals may be obtained from Bailey *et al.* (1999). In this study, the possible use of an *Acetobacter xylinum* biofilm as an adsorbent for mercury ion from aqueous solutions was studied. The effect of pH on the biosorption capacity was characterized by measuring the adsorption isotherms of mercury with the biofilm under pH controlled conditions. The maximum capacity of the biofilm, based on dry weight, was determined by varying the concentration of the mercury in the adsorption medium.

MATERIALS AND METHODS

Biofilm Production

The *Acetobacter xylinum* NBRC 13693 used in this study was obtained from the NITE Biological Resource Center (NBRC), Japan. The stock culture was grown in SH medium at 28°C under static culture conditions. The SH medium was composed of 2% (W/V) glucose, 0.5% (W/V) Yeast extract, 0.5% peptone, 0.27% (W/V) Na₂HPO₄ and 0.115% (W/V) citric acid (Romling, 2002). After cultivation, the cellulose sheets were removed and rinsed with distilled water and divided from bacterial and medium residues by 2% sodium dodecyl sulfate and 4% NaOH solutions in a boiling water bath. The biofilm was then dried at 70°C.

Biofilm Characterization

The surface morphology of an *Acetobacter xylinum* biofilm was studied by scanning microscopy (XL30 Philips model). The specific surface area was determined using the multiple BET method (Micromeritics, Gemini) with nitrogen gas as the adsorbate.

Adsorption Experiments

All adsorption experiments were carried out in batch conditions. Mercury was prepared from a synthetic solution (50 mL) of predetermined concentration, which was taken separately into 100 mL stoppered conical flasks. The pH of the working solutions was adjusted to the desired value using HCl and NaOH. The mercury concentration was analyzed following the cold vapor atomic absorption method at 253.7 nm (UNICAM, model 929, UK). The calibration curves were prepared by measuring the absorbance of a series of standard solutions at known mercury concentrations. A measured quantity of the adsorbent was added to the mercury solution and shaken in a mechanical shaker for a definite period of time. The difference in the adsorbate content before and after adsorption represented

the amount of adsorbate adsorbed by the *Acetobacter xylinum* biofilm. In order to test the effect of pH on the adsorption capacity, samples were adjusted at different pH ranging between 2 and 12 using 0.1 M NaOH and HNO₃. The period of contact was varied from 0.0 to 120 min. To describe the adsorption kinetics of mercury by the biofilm, adsorption isotherms of the Freundlich and Langmuir models were used.

Desorption Studies

The mercury loaded biofilm was regenerated with 1 M HCl solution on a rotary shaker at 100 rpm and the regenerated biofilm was reused in the next cycle with mercury solution from the adsorption experiments.

RESULTS AND DISCUSSION

Acetobacter xylinum Biofilm

The thick, gelatinous membrane formed in static culture conditions. The structure, not found in plant cellulose, results in high cellulose crystallinity (60-80%) and enormous mechanical strength (Astley *et al.*, 2001). 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. *Acetobacter xylinum* is an important industrial microorganism used in the production of bacterial cellulose (Geyer *et al.*, 1994). It is a simple gram negative bacterium which has an ability to synthesize a large quantity of high-quality cellulose organized as twisting ribbons of microfibrillar bundles (Shah and Brown, 2005). During the biosynthesis process, various carbon compounds of the nutrition medium are utilized by the bacteria, then polymerized into single, linear β -1-4-glucan chains and finally secreted outside the cells through a linear row of pores located on their outer membrane. Particularly impressive is the fact that the size of the microbial cellulose fibrils is about 100 times smaller than that of plant cellulose (Shirai *et al.*, 1997). This unique nano morphology results in a large surface area that can hold a large amount of water and at the same time displays great elasticity, high wet strength and conformability. The small size of the microbial fibrils seems to be a key factor that determines the microbial cellulose's remarkable performance as an effective adsorbent. Unlike cellulose of plant origin, microbial cellulose is entirely free of lignin and hemicelluloses (Lynd *et al.*, 2002). A vigorous treatment with strong bases at high temperatures allows the removal of cells embedded in the cellulose net and it is possible to achieve a high performance absorbent. The weight loss in the pellicles as a result of alkaline treatment was found to be around 15-20%, which is attributed to the loss of protein and nucleic acid contents (Shirai *et al.*, 1997). The specific surface area of the biofilm determined by the BET method was $650 \pm 0.08 \text{ m}^2 \text{ g}^{-1}$.

Effect of pH

The effect of pH on the adsorption capacity is characterized by measuring the mercury adsorption to the biofilm of *Acetobacter xylinum* under pH controlled conditions. The maximum mercury adsorption occurred at around neutral pH. Under acidic conditions the adsorption of mercury by the biofilm was quite low (Fig. 1). Normally, metal adsorbents exhibit a drastic decrease in metal affinity at low pH conditions. The occurrence of competition between protons and metal ions for the same sites should also be considered particularly at low pH values. There was an increase in mercury adsorption per unit weight of the biofilm with increasing pH, from pH 3.0 to 5.0. This phenomenon seems to level off at pH greater than 8.0. The increase in adsorption levels observed with increasing pH can be explained by the strong relation of adsorption to the number of surface negative charges, which depends on the dissociation of functional groups. It can also partly explain the low amounts of metal ions retained by the adsorbent at pH values below 4, as most functional groups are expected to

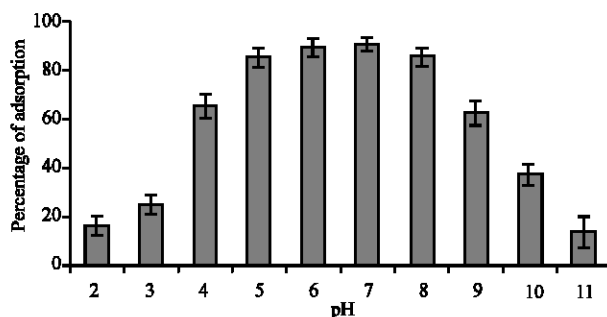


Fig. 1: Adsorption of mercury on the biofilm as a function of pH. Conditions-contact time: 50 min; biofilm mass: 2 g; mercury: 100 mg L⁻¹; temperature: 20°C

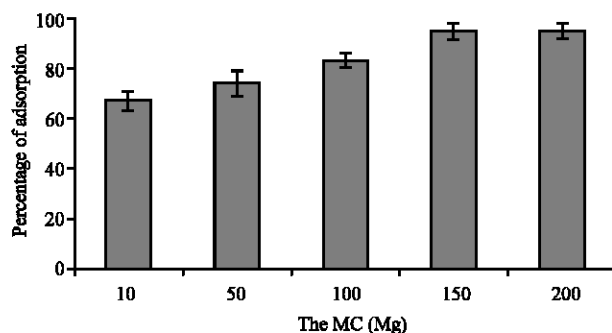


Fig. 2: Percentage of mercury ion adsorption on the biofilm as a function of amount of adsorbent. Conditions-contact time: 50 min; mercury: 100 mg L⁻¹; temperature: 20°C

dissociate only at neutral pH values. The effect of pH on the biosorption of Hg (II) was investigated by Chang and Hong (1995). Their results showed that biosorption of Hg (II) by non-living *Pseudomonas aeruginosa* PU21 was pH dependent and that maximum biosorption was obtained at pH 7.4. Chen *et al.* (1997) reported that the pH profile of Hg (II) bioaccumulation by recombinant living *E. coli* remained at the same level within a pH range of 3.0-9.0. Their results demonstrate that, in contrast to biosorbents or ion exchange resins, the Hg (II) bioaccumulation system was resistant to pH over a broad range.

Effect of Adsorption Time

Adsorption equilibrium was established around 50 min, after which the adsorbed mercury did not significantly change further with time. Several parameters affect the biosorption rate. These include: structural properties of biosorbent (e.g., protein and carbohydrate composition and surface charge density, topography and surface area), amount of adsorbent, properties of the ions under study, stirring rate, initial concentration of ionic species and the presence of other metal ions.

Effect of Adsorbent

The increase of the biofilm leads to the enhancement of percent removal of mercury (Fig. 2). The result of the present study falls in line with the findings of Manohar *et al.* (2002). The capacity of the biofilm for adsorption of mercury is the same order of magnitude as the heavy metal ion adsorption capacities of the various biomasses and adsorbents (Kacar *et al.*, 2002; Saglam *et al.*, 2004). Chen *et al.* (1997) used a living *E. coli* strain for bioaccumulation of mercury, where the highest bioaccumulation level was about 17.6 mg g⁻¹ dry biomass. Hafez *et al.* (1997) reported that the

adsorption capacity of *Aspergillus flavus* was 46 mg for uranium per g of dry biomass. The adsorption capacity of *Rhizopus arrhizus* was 78 mg for Fe (III), 71 mg for Pb (II) and 62 mg for Cd (II) per g of dry biomass (Ozer *et al.*, 1997). Other than microbial biomass, various sorbent materials have been used for removal of heavy metal ions from industrial waste, ranging from natural polysaccharide gels to coal and functionalized synthetic polymers (Namasivayam and Kadirvelu, 1999; Manohar *et al.*, 2002). In these systems, the metal removal process is based on a solid-liquid contenting and separation process. Such preparations offer advantages in terms of mechanical strength and durability, handling and ease of scale up. For example, Binman *et al.* (1997) used sulphur-chlorinated jajoba wax attached to polystyrene beads, for which the maximum inorganic mercury adsorption capacity was 50 mg g⁻¹. Jyo *et al.* (1997) prepared phosphoric acid treated poly (glycidyl methacrylate-co-divinyl benzene) beads, for which the mercury adsorption capacity of the sorbent was 40 mg g⁻¹. Unfortunately, all of the above mentioned sorbents are expensive and require several steps for preparation. In contrast, the adsorption capacity of the biofilm reached 180 mg g⁻¹ with mercury, a value comparable with the values reported in the previous studies. It is larger than the adsorption capacity of well known adsorbents such as coir pith carbon (154 mg g⁻¹) (Namasivayam and Kadirvelu, 1999), PHC-peanut hull carbon (110 mg g⁻¹) and granular activated carbon (124 mg g⁻¹) (Namasivayam and Periasamy, 1993). Ma *et al.* (1992) report a substantially lower value (0.8 mg g⁻¹) for a granular activated carbon.

Effect of Hg (II) Concentration

The maximum percentage of mercury was adsorbed at lower initial concentrations. The percentage mercury adsorption was 91% at a concentration of 80 mg L⁻¹. A further increase in initial concentration decreased the adsorption percentage (e.g., an initial concentration of 120 decreased adsorption to 75%). At low concentrations of mercury, the ratio of sorptive surface area to total metals ions available is high and thus, there is a greater chance for metal removal and the removal capacity is higher.

Sorption Isotherms

The experimental data of mercury sorption by biofilms of *A. xylinum* were fitted to the Langmuir and Freundlich models. The Langmuir model is applied to the experimental equilibrium data (Yu *et al.*, 2003).

$$Q_e = \frac{bQ_0C_e}{1 + bC_e}$$

where, Q_e is the amount of mercury adsorbed at equilibrium (mg g⁻¹); C_e is the equilibrium concentration (mg L⁻¹); Q_0 is the adsorption capacity (mg g⁻¹) and b is the energy of adsorption (Langmuir constant). The linear plot of the isotherm of the equation is an obligatory condition for the proper application of the theory of monomolecular adsorption. The linear plot of $C_e = q_e$ versus C_e shows that the description of the adsorption of mercury on the adsorbent by the Langmuir isotherm model is satisfactory. Q_0 and b are determined from the slope and intercept of the plot. Some theoretical parameters in the Langmuir equation were calculated from the experimental data. The linear form of the Freundlich equation (Slejko, 1985) is applied to the results of adsorption.

$$\text{Log}_{10} \frac{x}{m} = \text{Log}_{10} K_f + \frac{1}{n} (\text{Log}_{10} C_e)$$

where, x is the amount of the adsorbed solute (mg), m is the weight of the adsorbent (g), C_e is the equilibrium concentration (mg L⁻¹), $1/n$ is the slope, showing the variation of the adsorption with concentration and k_f is the intercept, showing the adsorption capacity of the adsorbent. Linear plots of $\log_{10} (x/m)$ versus $\log_{10} C_e$ show that the adsorption follows the Freundlich isotherm model. The values between 1 and 10 represent beneficial adsorption. The adsorption isotherm of mercury on the

biofilm can be included in type L on the Giles classification (AnoopKrishnan and Anirudhan, 2002). Langmuir's isotherm is based on the assumption that points of valency exist on the surface of the adsorbent and that each of these sites is capable of adsorbing one molecule; thus, the adsorbed layer will be one molecule thick. Furthermore, it is assumed that all the adsorption sites have equal affinities for molecules of adsorbate and that the presence of adsorbed molecules at one site will not affect the adsorption of molecules at an adjacent site. Freundlich's isotherm is based on the assumption that the adsorbent has a heterogeneous surface composed of different classes of adsorption sites, with adsorption on each class of site following the Langmuir isotherm.

Desorption Experiments

In order to evaluate the reusability of the biofilm, the regeneration and consecutive adsorption cycles were carried out using 1 M HCl. The results showed that the adsorption efficiency for the biofilm regenerated with 1 M acid, decreased to 43% for the first regeneration cycle and to 61% for the second treatment.

CONCLUSION

The results presented here indicate that a biofilm prepared from *Acetobacter xylinum* is a suitable and effective adsorbent for the removal of mercury from aqueous solutions. The adsorption of mercury on the adsorbent depends on the experimental conditions, particularly on the pH of solution and the metal concentration ion in the medium. The good fit of the adsorption isotherm for mercury was observed for Freundlich and Langmuir models. The calculated values of the Freundlich and Langmuir constants indicated that the mercury adsorption onto the *Acetobacter xylinum* biofilm was a function of initial mercury concentration.

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