Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioresource Technology 97 (2006) 1907–1911
BIORESOURCE TECHNOLOGY

Mercury(II) removal from aqueous solutions by nonviable *Bacillus* sp. from a tropical estuary

Carlos Green-Ruiz *

Mazatlán Academic Unit, Institute of Marine Sciences and Limnology, National Autonomous University of Mexico, Av. Joel Montes Camarena s/n, Col. Centro, 82000 Mazatlán, Sin., México

Received 29 October 2004; received in revised form 5 August 2005; accepted 17 August 2005
Available online 10 October 2005

Abstract

Use of microorganisms for removing mercury is an effective technology for the treatment of industrial wastewaters and can become an effective tool for the remediation of man-impacted coastal ecosystems with this metal. Nonviable biomass of an estuarine *Bacillus* sp. was employed for adsorbing Hg(II) ions from aqueous solutions at six different concentrations. It was observed that 0.2 g dry weight of nonviable biomass was found to remove from 0.023 mg (at 0.25 mg L^{-1} of Hg(II)) to 0.681 mg (at 10.0 mg L^{-1} of Hg(II)). Most of the mercury adsorption occurred during the first 20 min. It was found that changes in pH have a significant effect on the metal adsorption capacity of the bacteria, with the optimal pH value between 4.5 and 6.0 at 25 °C when solutions with 1.0, 5.0 and 10.0 mg L^{-1} of Hg(II) were used.

Keywords: Biosorption; Bacteria; Bioremediation; Isotherms; Mercury sorption

1. Introduction

Mercury is a pervasive contaminant that is highly toxic and is readily accumulated by organisms. According to Manohar et al. (2002) the major effects of mercury poisoning are neurological and renal disturbances as well as impairment of pulmonary function. Natural inputs of mercury to the environment are related to weathering of mercuriferous areas, the degassing from surface water and from the earth's crust through volcanic eruptions, naturally-caused forest fires, and biogenic emissions (Morel et al., 1998). In addition, this metal is also released to the environment from anthropogenic activities that include agriculture, battery production, fossil fuel burning, mining and metallurgical processes, paint and chloralkali industries, and wood pulping (Boening, 2000).

Estuaries encompass a great variety of habitats that include mangrove forest, salt marshes, intertidal pools, swamps; all of them possess high biological diversity and a rich and complex food chain (Flores-Verdugo et al., 1996). These ecosystems constitute important fishery and nursery areas encouraging human settlements (Páez-Osuna et al., 1998). Like most of the coastal zones, estuaries receive heavy metals from the anthropogenic activities developing on their drainage basin, and they act as temporal sinks for these contaminants. For instance, von Canstein et al. (1999) reported concentrations from 1.6 to 7.6 mg Hg L^{-1} for effluents from several chloralkali factories in Europe. Eventually, this kind of industry pours its wastewater into rivers, which transport mercury to coastal zones. Typical background mercury concentration for uncontaminated coastal sediments ranged from 0.39 to 1.62 μg g^{-1} (Kot et al., 1999); nevertheless 27.53 μg g^{-1} have been found in sediments from Kastela Bay, Croatia (Kwokal et al., 2002). Moreover, elemental mercury is efficiently transported as a gas, affecting the most remote areas of the globe (Morel et al., 1998). Thus, the development of clean-up technologies for the treatment of water, sediments, and soils contaminated with this metal are of major concern.

* Tel.: +52 669 985 2845; fax: +52 669 982 6133.
E-mail address: cgreen@ola.icmyl.unam.mx

0960-8524/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2005.08.014

According to Gazsó (2001), conventional techniques for removing toxic metals (e.g. ion exchange, precipitation, electrochemical) may not be effective, especially when the metal concentration is lower than 100 mg L^{-1} (Volesky, 1990a). However, the use of microorganisms in the removal of metals from contaminated wastewater, mining, and industrial wastes is generally considered promising (Nourbakhsh et al., 2002). Mercury reduction to its metallic form by a *Pseudomonas putida* strain and subsequent metal volatilization has been suggested by von Canstein et al. (1999), while mercury sulfide-mediated precipitation through the activity of sulfate-reducing bacteria has been reported by King et al. (2002). In addition, Hassen et al. (1998) have studied the sorption of mercury from aqueous solutions by *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. Microbial cell surfaces are usually charged and the passive process involving extracellular accumulation of metals on cell walls has been studied (Sahoo et al., 1992). Inactivated, nonliving microbial biomass can serve as a basis for the development of potent biosorbent materials of strategic or valuable heavy metals and has a potential application in environmental control of these toxicants (Volesky, 1990a). In order to determine the feasibility of using nonviable biomass of *Bacillus* sp., which is indigenous from Urías Estuary (mouth of the gulf of California), it was examined as an adsorbent to mercury removal. In this study, biosorption batch experiments were performed for evaluating: (1) its metal sorption capacity, and (2) the effects of various mercury concentrations and pH values on this process.

2. Methods

2.1. Microorganisms

As a part of a previous research focused on Mn(II) oxidation by indigenous bacteria, several strains were isolated from sediment collected from the Urías estuary, Sinaloa, Mexico. Pure cultures were obtained by subsequent inoculations to nutrient plates from an enrichment culture and their capacities of oxidize Mn(II) were evaluated (Green-Ruiz, unpublished data). A pure culture was selected to be used as the test organism because of its higher Mn(II) oxidation capacity. The same culture was chosen to carry out the present Hg(II) adsorption experiments. Microscopic and biochemical analyses were performed and results indicated that the culture belongs to the *Bacillus* genus. It is a rod-shaped endospore-forming, aerobic, and Gram-positive bacterium, which produces cytochrome oxidase, does not break down tryptophan, cannot use citrate as its only source of carbon, and ferment carbohydrates but not glucose and sucrose (Rodríguez-Tirado, unpublished data). *Bacillus* sp. was cryopreserved (−70 °C) on pre-sterilized glass bead and an inoculation was made when it was needed for experiments.

The *Bacillus* sp. used here has been submitted to the Collection of Aquatic Important Microorganisms (CAIM), and has been assigned key number CAIM 726. CAIM is registered in the Word Data Center on Microorganisms of the World Federation for Culture Collections as number WDCM813.

Bacteria were cultured in liquid medium K, which was prepared by pouring 0.5 and 2.0 g of yeast extract and peptone, respectively, into two-L flasks, which contained 750 mL of seawater (salinity about 34) and 250 mL of Milli-Q purified water. Flasks were incubated on an orbital shaker at 100 rpm at 25 °C for 3 days. Biomass was harvested from the medium by centrifugation at 5000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged. Microscopic observation of bacteria showed that bacteria were not lysed at this point. The biomass was autoclaved at 121 °C for 20 min and plate count tests were performed in order to ensure that nonviable cells were obtained. The biomass was then re-suspended in 10 ml of purified water and, after stirring, a 0.5 ml-aliquot was withdrawn and dried at 60 °C for 8 h in a conventional oven and it was weighed. Calculations were done to dilute the biomass solution with purified water until a concentration of 400 g L^{-1} was reached. Finally, the biomass solution was stored at 4 °C into a plastic vial until the experiments were conducted (no more than 2 days).

2.2. Mercury removal

Nonviable biomass was employed directly for Hg(II) removal batch experiments. Mercuric chloride ($HgCl_2$) and Milli-Q purified water were used to prepare Hg(II) solutions at different concentrations (0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 mg L^{-1}). The volume of biomass solution equivalent to 0.2 g dry weight of biomass (0.5 mL) was put into 250 ml polypropylene flasks containing 100 ml of mercury solution with six different initial concentrations and a mercury-free control, and agitated on an oscillating shaker at 100 rpm for a period of 120 min at room temperature (25 °C). The volume of biomass solution added to the metal solutions represents the 0.5% of the total volume. Hydrochloric acid and sodium hydroxide were used for adjusting the initial pH of all the metal solutions to six. No additional pH measurements were made during the experiment. Three-ml samples were taken from each flask at the beginning and the end of the experiments. The samples were filtered through 0.22 μm-nitrocellulose filters and filtrates were analyzed for Hg concentration with a mercury analyzer cold vapor Buck Scientific Model 400A.

In addition, mercury solutions with concentrations of 1, 5 and 10 mg Hg L^{-1} were used to investigate the pH effect on the mercury removal capacity of the nonviable biomass in the pH range from 3 to 9. All of the material was acid-washed (Moody and Lindstrom, 1977) prior to use. In order to identify any contamination due to the material and procedure used in the experiments on the actual concentration of mercury we measured, two blanks were run