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Investigation on batch biosorption of lead using *Lactobacillius bulgaricus* in an aqueous phase system

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Abstract

In this research, the biosorption of lead by *Lactobacillus bulgaricus* was investigated. The mechanism for the biosorption was similar to ionic exchanger. The media pH, weight of dried biomass and initial lead concentration were investigated at ambient temperature. At high acidic solution (pH<1.5) and also in alkaline condition, there was no significant absorption. In these experiments the highest biosorption of lead occurred at pH value of 6 and the biomass concentration of 4.5 g/l. The removal efficiency of 88.8 percent was obtained with initial lead concentration of 50 mg/l. It was found that *Lactobacillus bulgaricus* was highly efficient in low ion concentration (less than 100ppm), however for the lead concentration higher than 100ppm the percent of removal was low (less than 50 percent).

Keywords: *Lactobacillus bulgaricus*, biosorption, lead, dried biomass

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INTRODUCTION

The advanced technologies for the industrialization and urbanization have substantially enhanced the degradation of our aquatic environment through the discharge of industrial waste waters and domestic wastes[1,2]. Heavy metal pollution has become one of the serious environmental problems of worldwide concern because of their extreme toxicity and tendency for bioaccumulation in the food chain even in relatively low concentrations[3,4].

The toxic heavy metals cause serious threat to the environment, animals and humans[5]. In 2000, the United States Environmental Protection Agency (USEPA) prepared a list of organic and inorganic pollutants found in wastewater and which constitute serious health hazards[6]. Lead is one of the metals on the list and it is extremely toxic to organisms even at low concentration. It can damage the nervous system, gastrointestinal track, encephalopathy with pretreatment damage, kidneys and reproductive system particularly in children[1,7].

Exact determination of heavy metal ions in the environmental samples by instrumental techniques like graphite or flame atomic absorption spectroscopy and inductively coupled plasma optical emission spectroscopy is an important in the field of analytical chemistry[8]. Various processes and measurements such as chemical precipitation, ion exchange, membrane separation and activated carbon adsorption have been employed to remove metal pollutants from aqueous solutions[9,10]. However most of these methods suffer from some disadvantages including incomplete metal removal, toxic sludge generation and high costs when applied to dilute effluents on a large scale. These lead to a search for cheaper, easily obtainable materials for adsorption of heavy metals or organic pollutants[11,12].

Biosorption is a rapid, reversible, economical and environmentally friendly technology in contrast to traditional methods used for removal of heavy metals from streams[13-16]. The mechanism of binding by inactivated biomass may depend on the chemical nature of pollutant (species, size and ionic charge), type of biomass, its preparation and its specific surface properties and environmental conditions (pH, temperature, ionic strength, existence of competing organic or inorganic ligand in solution). Although biosorption is generally used for the treatment of heavy metal pollutants for wastewaters, it can also be considered a promising technology for the removal of organics from industrial waste streams and polluted natural waters[17-19].

The objective of this work was to investigate the effect of initial concentration of lead, pH and biomass concentration on the biosorption of lead using *Lactobacillius bulgaricus* as biomass.

MATERIALS AND METHODS

Lactobacillus bulgaricus was supplied by Biotechnology Research Center, Noshirvani University of Technology, Babol, Iran. The organism was originally isolated from whey. The microorganism was grown aerobically in an agitated vessel (100 rpm). The medium consisted of 2g/l yeast extract, 20g/l glucose, 0.2g/l K_2HPO_4, 0.2 g/l $MgSO_4$ and 0.05 g/l $MnSO_4$. The medium was sterilized, autoclaved at 15psig and temperature of 121°C for 20 min. The pH of medium was initially adjusted at 6.5 and the inoclum was introduced after which the culture was incubated at 30°C for 48 hours and the biomass was harvested at maximum cell growth. The filtration process was accomplished using cellulose acetate filter with pore size of 0.45 μm (Sartorius Biotech, Germany). The harvested cells were washed with deionized water to improve the metal binding properties and the biomass was dried at 105°C in an oven for 24 hours[20]. Finally, the biomass was milled and mechanically sieved to give uniform particles in the range of 250-800μm.

All chemicals and reagents used for the experiments were analytical grades and supplied by Merck (Germany). The pH meter, HANA 211(Romania) model glass-electrode was employed for measuring pH values in the aqueous phase. The initial pH of the working solutions was adjusted by addition of HNO_3 or NaOH solution, except for the experiment examining the effect of pH to be active. Fresh dilution was used for each set of the sorption experiments. The stock solution of Pb^{2+} (1 g/l) was prepared by dissolving $Pb(NO_3)_2$ in deionized water.

Batch biosorption experiments were carried out in flasks at 120rpm for the period of contact time using a heating stirrer (VELP Scientifica, Italy). The metal ion concentration was measured in triplicate by flame atomic absorption spectrophotometer (Philips, PU 9400, USA).

Scanning electron microscope

Scanning electron microscope (SEM) was used to show the texture and exposure surface of the dried biomass *Lactobacillius bulgaricus*. The samples were dried by liquefied nitrogen, coated with gold and observed with a (Phillips XL30, Holland) microscope. Finally, image of the samples were taken under SEM at x10000 magnification[21].

RESULTS AND DISCUSSION

The morphology of the dried biomass *Lactobacillius bulgaricus* examined by SEM is presented in Figure 1. The obtained SEM image demonstrated *Lactobacillius bulgaricus* biomass was cylindrical in shape. The created surface had great potential to adsorb heavy metals.

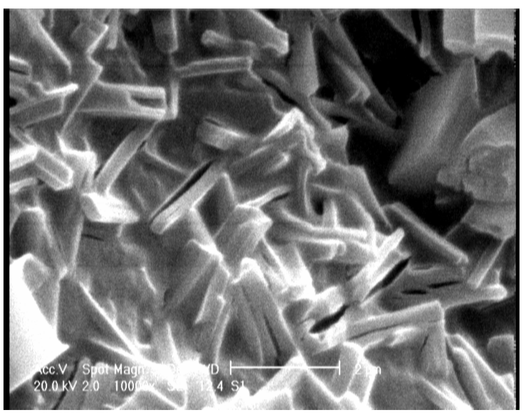

Figure 1: Scanning electron microscopy of the outer surface of the *Lactobacillius bulgaricus* at x10000

The pH is one of the influential parameters which affect biosorption capacity of the heavy metals[22,23]. It is well known that at strong acidic conditions (pH of less than 1.5) nearly no lead adsorption is observed[24]. Acidity pH of the aqueous system also influenced the capacity of the sorbent to take up the heavy metal ions, because pH is known to affect the solution chemistry of the metal ions when they are hydrolyzed. Hydrolyzed metal ions tend to form complex organic or inorganic ligands during hydrolysis and condensation reaction catalyzed by an acid or base.

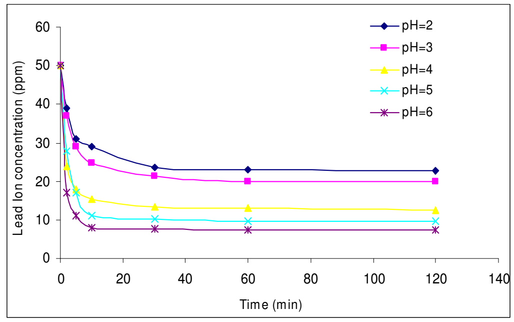

Figure 2: Effect of pH on biosorption capacity at various lead concentrations and biomass concentration of 4g /l

Figure 2 presents the effect of pH on biosorption capacity. As the pH increased in the solution, higher uptake of lead occurred. When the pH was increased from 1 to 6, the biosorption capacity was increased from zero to 42.6 mg/g for the initial lead concentration of 50 mg/l. The removal efficiency of lead with respect to pH is shown in Figure 3. At pH of 6, an 85.2 % biosorption of the intial concentration of lead was achieved.

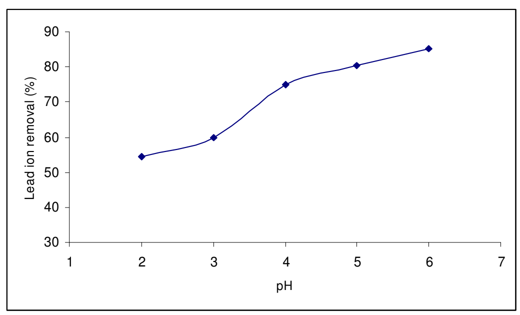

Figure 3: Lead ion removal at various pH and at 298°K

At alkaline pH of 8 and higher, the lead ions had precipitated (data not shown). The best pH range for biosorption occurred at 4-6. There was strong resistance against the metal ion adsorbed to the biomass surface probably because functional groups in biomass were highly protonated at strong acid condition since negatively charged groups at biosorbent surface is necessary for the sorption process[25]. Such sites were unavailable due to competition between Pb^{2+} and H_3O^+ ions when the pH is less than 1.5. When the acidity decreased in the solution, the deprotonation of acid functional groups, such as carboxyl, phosphonate and phosphodiester, were strengthened[22-25].

The effect of biomass concentration on lead ion removal was also experimented. A small amount of biomass had the potential to remove the heavy metals from an industrial effluent. In order to use minimum amount of biomass for the maximal removal of lead, different weight of biomass from 2 to 5 g/l were used.

Results in Figure 4 show that the maximum biosorption capacity occurred at biomass concentration of 4.5g/l.

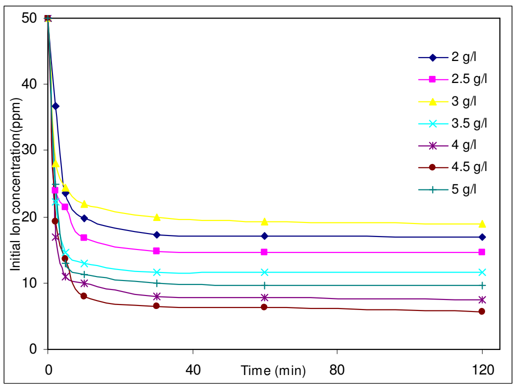

Figure 4: Effect of biomass concentration on lead ion removal at pH 6 and lead initial concentration of 50mg/l

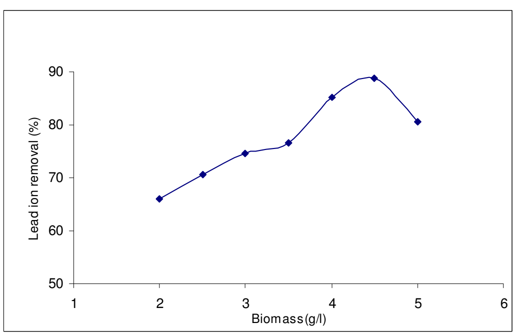

Figure 5: Effect of biomass concentration on lead removal at pH 6

The percentages of the lead removal by biomass concentration of 2 to 5g/l with an increment of 0.5g/l were 66.0, 70.6, 74.6, 76.6, 85.2, 88.8 and 82.4, respectively. The data are presented in Figure 5. By increasing the concentration of the biomass, the lead ion removal was increased.

Additional biomass more than 4.5 g/l may decrease the heavy metal removal.

A set of experiments conducted with lead concentration, in the range of 20, 70, 120, 170, 220 mg/l were prepared to determine the biosorption data at optimum condition (pH of 6 and dried biomass concentration of 4.5 g/l). The results are shown in Figure 6.

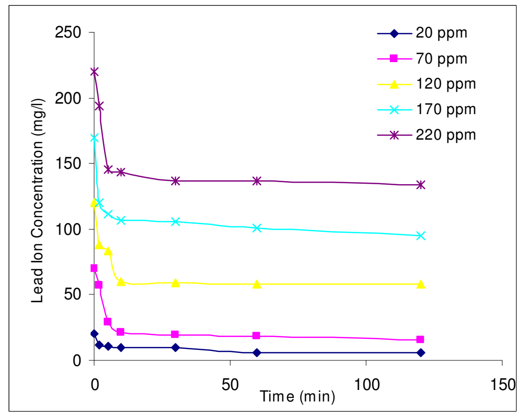

Figure 6: Lead concentration profile with respect to time

The lead uptake was increased with the initial metal ion concentration. Ironically, it was found that the sorption process was active in early stage and most of the lead ion concentrations were absorbed within 30 minutes.

Figure 7 shows the percentage of lead removal at various lead ion concentrations. The lead removal at low ionic concentration was initially increased till 50 mg/l. Clearly, the percentage of removal was decreased at high lead ion concentrations.

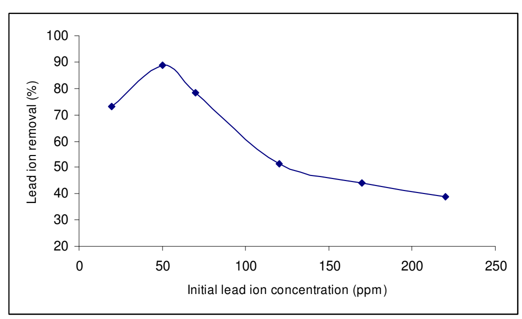

Figure 7: Lead removal with respect to lead initial concentration

The results show that *Lactobacillius bulgaricus* biomass was an interesting biomaterial with a low cost biosorbent for the lead removal from an aqueous solution, especially at low ionic concentration of about

50mg/l. The biosorption process was pH dependent, as the pH increased from 2 to 6 the lead removal was increased from 54.4 to 85.2 percent, respectively.

The removal efficiency was also increased with an increase of biomass concentration of 4.5 g/l. The metal ion concentration increased from 20 to 220mg/l, the removal was decreased. There was no biosorption at highly acidic and alkaline conditions. At near neutral condition, the biosorption showed to be effective.

We conclude that biosoption of lead at concentration of 50 mg/l by *Lactobacillus bulgaricus* at biomass concentration of 4.5 g/l is fastest at pH 6 as the efficiency of removal of lead was 85.2% and we suggest that *Lactobacillus bulgaricus* can be useful for biosorption of lead from an aqueous system

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