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Characterization of metal removal of immobilized *Bacillus* strain CR-7 biomass from aqueous solutions

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ABSTRACT

Bacillus strain CR-7 of multiple metal and antibiotic resistances was isolated. Its metal adsorption under different pretreatments and immobilizations from aqueous solution was characterized. Pretreatment with NaOH (0.1 mol L^{-1}) significantly improved Cu^{2+} adsorption capacity of the bacterial biomass. Sodium alginate (2%) was the ideal immobilization matrix. The immobilized and pretreated biomass had an obvious "orderliness", following the order of $Cu^{2+} > Zn^{2+}$ in the solution containing these two metals, and following the order of $Pb^{2+} > Al^{3+} > Cr^{6+} > Cu^{2+} > Fe^{3+} > Zn^{2+} = Ni^{2+} > Cd^{2+} = Co^{2+} > Mn^{2+}$ in the solution containing these 10 metals. ΔH° and ΔS° of Cu^{2+} adsorption were +7.68 J/mol and +16.628 J/mol K, respectively. The infrared peak of –N–H shifted greatly after Cu^{2+} adsorption. After adsorption treatment, some molecular groups disappeared in un-immobilized biomass but were still present in the immobilized biomass. Cu^{2+} adsorption fit both Langmuir and Freundlich isotherm models. It was concluded (1) that the Cu^{2+} adsorption process was endothermic, (2) that –N–H is a most important Cu^{2+}-binding group, (3) that immobilization prevents loss or damage of the Cu^{2+}-binding molecular groups, and (4) that Cu^{2+} adsorption of pretreated and immobilized biomass is homogeneous.

1. Introduction

Growing amount of heavy metal-polluted wastewater is rooted in the aggressive industrialization and urbanization [1]. If no further treatment, the heavy metals in the wastewater are likely eventually absorbed by and accumulated in living organisms, and threaten health of the living organisms. Even if some trace elements such as copper are essential to growth and development of the living organisms, they have toxic effects on the living organisms at high concentrations [2]. The polluted wastewater has therefore received much concern [3]. Methods on treatment of heavy metal-polluted wastewater can be divided into (1) physical and/or chemical reactions such as chemical precipitation, ion exchange, filtration, and (2) adsorption either by biomaterials such as microbial and plant derived biomasses [4–6] or by non-biomaterials such as fly ash, carbon slurry, red mud, kaolinite, baggage fly ash, bentonite, electric furnace slag, and montmorillonite [7–10]. Physical and/or chemical reactions are not fast for removal processes but easy to commercialization and application. However, physical and/or chemical reactions-based methods have unacceptable defects such as high costs, high energy consumption, secondary pollution, and/or production of a large amount of toxic chemical sludge which is difficult to treat [5,11,12]. Relatively, adsorption methods have an advantage over physical and/or chemical methods because of low cost and low energy, and particularly due to no the secondary pollution [4,13]. However, if no other auxiliary measures, the microbial biomass-based treatment methods are hardly applied because of several major defects such as solid–liquid separation problems, possible biomass swelling, inability to regenerate/reuse, use in the continuous mode, and development of high pressure drop in the column mode [14,15]. To overcome these defects, immobilization techniques for microbial biomasses have been developed [14,16].

Most researches on immobilization techniques-based metal removal from the solutions that have been conducted were based on the use of granulized microbial adsorbents packed in columns [16]. This approach seems to be inapplicable to metal removal by free cells because the resulting bed is easily plugged by free cells

Abbreviations: AAS, atomic absorption spectrometry; CDC, Cu^{2+} adsorption capacity; CRE, Cu^{2+} removal efficiency; ΔH°, enthalpy change; ΔS°, entropy change; EDAX, energy dispersive x-ray analysis; IAS, infrared absorption spectra; IR, Infrared; PB, NaOH (0.1 mol L^{-1})-pretreated biomass; PVA, polyvinyl alcohol; rpm, revolutions per min; SEM, scanning electron microscope; UUB, un-pretreated and un-immobilized biomass.

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[16]. Therefore, microbial immobilization techniques still needs further study. Toward this goal, choice of the immobilization matrices is a key step. By now, numbers of immobilization matrices have been developed and used, such as sodium or calcium alginate, polysulfone, polyacrylamide, polyurethane and silica [14]. Even so, immobilization techniques specific to bacterial species are required not only because of bacterial species diversity [17] but also owing to difference in the nature of immobilization matrices.

Although *Bacillus* biomass has been used for removal of heavy metal from aqueous solution [14,16] researches on heavy metal removal by immobilized Bacillus biomass from the solutions were very limited. Immobilization matrices used in *Bacillus* biomass included Diaion SP-850 resin [18], silica gel [21], Amberlite XAD-4 [22], and calcium alginate [23]. However, little is known about characteristics of heavy metal removal by *Bacillus* biomass from aqueous solution with mixed metals, and effects of immobilization on metal-binding molecular groups of the biomass. In this study, *Bacillus* strain CR-7 was chosen as an adsorbent because of its resistance to multiple metals. The aim of this study was to characterize metal adsorption of the bacterial biomass immobilized with sodium alginate, gelatin, and polyvinyl alcohol (PVA).

2. Materials and methods

2.1. Isolation of bacteria

The soil sample from disposal sites of the tailings of a copper mine in Guangxi of China was used to isolate heavy metal-resistant bacteria. Briefly, the soil suspension prepared with sterile water was plated onto plates of Luria-Bertani's (LB) agar medium containing different concentrations of $CuSO_4{\cdot}5H_2O$. The plates were placed for incubation of bacteria for 24 h at 37 °C.

2.2. Analysis of metal and antibiotic resistance profiles of bacteria

One millilitre of overnight bacterial culture with an OD_{600} value of 0.2 was transferred onto 10 mL LB medium containing different concentrations of metal salts and antibiotics, and cultured for 24 h at 37 °C by rotation at 180 rpm. One hundred microlitres of the resulting bacterial culture were plated onto plates of LB agar medium without metal salts and antibiotics, and then placed for 24 h at 37 °C. The resistance was reported as maximum inhibitory concentration (MIC) that could inhibit growth of bacteria.

2.3. DNA manipulation and, amplification, cloning and sequencing analysis of bacterial 16S rDNA sequence

DNA manipulation referred to conventional methods in the literature [24]. Bacterial 16S rDNA was amplified from the bacterial genomic DNA as the method in the literature [25] by polymerase chain reaction with primers (forward 5′-TAGGGTTACC-TTGTTACGACTT-3′, and backward 5′-AGAGTTGATCATGGCTCAG-3′). The amplified DNA was cloned into the pUCm-T vector (Sangon, Shanghai, China) and then sequenced.

2.4. Treatment of bacteria

Two millilitres of overnight bacterial culture with an OD_{600} value of 0.5 were added to 200 mL LB medium, and cultured at 37 °C by rotation for 24 h at 180 rpm. The resulting bacterial culture was collected by centrifugation for 10 min at 4500 rpm. The collected bacteria were washed twice with sterile water until pH of the filtrate reached 7, and collected again by centrifugation.

For pretreatment, the collected bacterial biomass was added in NaOH, HCl or HNO_3 solution as a ratio 1 (g):100 (mL) of the bacterial culture to reagent, and incubated for 30 min at 35 °C by rotation at 150 rpm. The control treatment was conducted in sterile water in parallel with procedures of the pretreatment. All procedures for washing and collecting bacterial culture were performed as indicated above. The treated bacteria were collected and dried for 24 h at 80 °C. Dried bacteria were ground into powder and sieved through a 100-mesh sieve for further use.

2.5. Immobilization of bacteria

Powdered bacterial biomass was immobilized in sodium alginate, gelatin, and PVA. Immobilization procedures followed the methods in the literature [26]. Briefly, matrix solutions were sterilized as conventional moist heat sterilization method. Powdered bacterial biomass was mixed with the matrix solution as a ratio 1 (g):100 (mL). The mix was slowly dripped through a syringe with a needle 12 into 4% (w/v) $CaCl_2$ solution, generating immobilization beads. After laid aside for 24 h in $CaCl_2$ solution, the resulting immobilization beads were rinsed for further use with sterile water until pH of the washing effluent was up to 7.

The strength of immobilization beads was assayed as the method in the literature [26]. In brief, 50 beads were added to 100 mL water solution and subjected to rotation for 24 h at 220 rpm at 50 °C to observe breakage of the beads.

For assay of mass transfer resistance, immobilization beads were mixed with methylene blue dye as a ratio 5 (g):100 (mL) and laid aside for 24 h at room temperature. After that, the beads were fully rinsed with sterile water, and cut from the middle with a shaving blade to observe the internal coloring of the beads.

2.6. Batch experiment of metal adsorption

The metal adsorption was conducted as the rotation method in 100 mL solution containing metal (s). The conditions for adsorption were detailed in the figure legends. Quantitative analysis of metals in samples was conducted by atomic absorption spectrometry (AAS) on a Hitachi Z-8000 atomic absorption spectrophotometer (Tokyo, Japan) equipped with a graphite tube atomizer following standard procedures.

Energy dispersive x-ray analysis (EDAX) was performed for identification of metals using the Hitachi 3400-N scanning electron microscope (SEM) with the EDAX Genesis program and a SUTW-SAPPHIRE detector. Preparation of the samples, i.e. adsorbents, for SEM analysis referred to the procedures in the literature [27] but with some modifications. The samples were fixed in 0.1 mol L^{-1} phosphate buffer (pH 7.3) containing 2.5% glutaraldehyde for 30 min at room temperature, and then washed three times with the phosphate buffer followed by further dehydration in gradient concentrations of ethanol for 10 min each. The dehydrated samples were vacuum-dried. The dried samples were ground into powder, and then sputter-coated for scanning with gold. For parameters for instrument's operation, detector's inclination was 35°, spectra was registered from 0 to 20 keV with a resolution of 130.87 eV, and pulse reading time was 100 s.

The infrared absorption spectra (IAS) of the samples were recorded within the range of 4000–400 cm^{-1} with a resolution of 6 cm^{-1} on a Fourier transform infrared spectrometer (Nicolet 5700) with a DTGS detector. The samples were dried at 70 °C till constant weight and ground into powder. Each powdered sample (2 mg) was mixed with KBr (200 mg). The sample–KBr mix was pressed the mixture into a pellet, which was analyzed by the Fourier transform infrared spectrometer. The average over 64 scans was collected for each measurement.

Construction of adsorption isotherms referred to the methods described in the literature [19,28]. The thermodynamic parameters on the adsorption, enthalpy change (ΔH°) and entropy change (ΔS°), were assessed as the equations described in the literature [10].