Biosorption of lead and cadmium and the potential role of Shewanella oneidensis

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Abstract

The bacterium *Shewanella oneidensis* was isolated from the soil in southern of Iraq,and identified basis on the traditional tests (morphological and biochemicaly) and development way(high equrance gene sequence using 16S rRNA gene phylogeny analysis). The results, indicated that the isolates bacterium was *Shewanella oneidensis*. For emphasis the bioremediation ability of bacteria, advance techniques has been applied, included: Fourier Transform Infrared (FTIR), X-ray diffusion analysis (XRD) and broadcast electron microscope (TEM). Biosorption using different concentration from Cd^{+2} and Pb^{+2} with different times has been used to clarify the bioremediation ability. The results of TEM indicate the presence of these metals around the cells of this bacterium with the appearance of some morphological changes. The results of FTIR, and XRD showed a different shifting on the peaks of spectra when they compare with the normal one with occur different peaks for loading bacteria with studied metals, these changes can emphasize the absorption ability of these bacteria.

Keywords: *Shewanella oneidensis* ; Biosorption; FT-IR ; XRD ; TEM

Introduction

The removal of heavy metal from the environment using biological material knowing as biosorption process [1].Biosorption using live or dead organisms or their components is a promising biotechnology in the field of removing contaminants. The reason for this is due the abundance in biomass, simplicity and high efficiency, as well as its superiority over traditional methods. [2]. By the biosorbents the metal concentration can be decreased from ppm to ppb in the solution, It can effectively sequester dissolved metal ions out of dilute complex solutions with high adeptness and rapidly; therefore it is a perfect tools for the treatment of great volume and little concentration of difficult metals pollutants [3]. The behavior of biosorbents such as the metal ion isolator is the chemical synthesis of the microbial cells that make up them [4]. Although there are specific limitations to understand the mechanisms of biological absorption, they may be one or a combination of processes, such as “ion exchange, complexation coordination, adsorption, electrostatic interaction, chelation and micro precipitation” [3; 5].

Facultative *S. oneidensis* is ready to utilize different organic carbon(pyruvate, propionate, acetate, fumarate, and serine) as electron donors,in addition to its ability to reduce wide range of resolvable or hard compounds, such as iron III, manganese I, nitrate, nitrite, thiosulfate, trimethyl-amine N-oxide, fumarate, uranium and Cr (VI) [6; 7 and 8]. Many genes are involved In metal ions eductions,which include; mtrA, mtrB, and mtrC genes [9].
In line with these studied susceptibility, this bacterium underwent several studies, firstly interested on its handy respiration and its potential to engage in Co metabolic bioremediation of toxic metal oxide forms [8; 10; 11and 12]. Extensive work focus on study the ability of this bacterium in the bioremediation of soils contaminated with metals (13; 10;14 and 15].
The effects of humic acid on azo dye decolorization by *S. oneidensis* MR-1 were studied by Liu *et al*. [16] founded reduction of Pu (IV) to Pu(III) after 24 h. Renshaw *et al*. [17] condicted 3% of Pu was reduced and is cell-bound. Bretschger *et al*. [18] studied *S. oneidensis*, wild and some cytochrome mutants, current production and metal oxide reduction. Cruz Garcia *et al*. [19] studied respiratory nitrate ammonification by *S. oneidensis* MR-1. The result showed anaerobic cultures of *S. oneidensis* MR-1 grown with nitrate as the sole electron acceptor exhibited a sequential reduction of nitrate to nitrite and then to ammonium. Hong *et al.* [20] reported that azo dye decolorization by *S. oneidensis* S12 was accelerated by "humic substances acting as redox mediator. Boukhalfa *et al*. [21] also investigated the reduction of Pu (IV) by *S. oneidensis* with lactate as the electron donor. Middleton *et al*. [11] considered the reduction of Cr (VI) by *S. oneidensis* in the aerobic and denitrifying state and in the lack of an further presence of electron recipient [22*]*. The present work aims to ensure the ability of *Shewanella oneidensis to bioremediation of heavy metals by using highly development* characterization *technology* like FT-IR,XRD and TEM to emphasize the presence of Pb and Cd round the bacterial cell.

Materials and Methods

Source of bacterial isolate

Shewanella oneidensis applied in the present work was isolated from the Um - Qasr district, southern Iraq. For bacterial identifying, biochemical tests and sequencing 16S rRNA gene has been depending .Result of 16sRNA then compared with the database in the NCBI GenBank .

Biosorption practice

Batch experiments where performs to study the biosorption ability of biosorbent *S. oneidensis*

Biosorbent description

The Fourier Transform Infrared test

Characterization of biosorbent before and after loading with Lead and Cadmium was carried out using FT-IR (Perkin Elmer spectrometer model 100 series).

X-ray powder diffraction analysis (XRD):

The characterization study using XRD analysis was done to characterize the biosorbent before and after loading with Lead and Cadmium by using the diffractometer analysis(Shimadzu 6000 series).

Transmission Electron Microscope:

The broth culture of bacteria was centrifuged for ten minutes at a force 3000 rpm, and emptying the floating part, protective the bacterial cell with 4% glutaraldehyde until four hours at 4°C. Sample then break away, emptying fixing substance and adding an adequate amount of bodily plasma, then letting serum clotting. Repeat the wash with 0.1M of Cacodylate buffer three times. Osmium tetroxide (1%) then use to fixing the sample oven night at 4°C. The sample is washed again with the same washing buffer for. The successions of acetone concentration included (35, 50,75, 95,and100%) was used for dehydration (Table1).

.Table 1: The sample was infiltrated with acetone and resin

Acetone		Resin	time
1	:	1	h
1	:	3	2h
		100% resin	Overnight
		100% resin	2h

Results and Discussion

FT-IR

Infrared spectra of *S. oneidensis* un- filled and filled with Cadmium (II) and Lead (II) ions alalysied to search for functional groups,which responsible for sorbtion both of them (Fig. 1).

This figure displays a number of absorption peaks, indicating the complex nature of the biomass. The spectra of un-filled biosorbent shows wide squad at 3247 cm^{-1} because of shifted the O-H group to 3245 and 3242 cm^{-1} for the loaded with Pb(II) and Cd (II), respectively and this may be due to complexation of O-H groups with these metals [23]. Complexation of carbonyl (C=O) group with a metal ions was responsible for peaks shifted from 1640 cm^{-1} to low frequency and appeared at1637 and 1633 cm^{-1} respectively, after loaded with Pb (II) and Cd (II)[24].While interaction of organic phosphate group with Pb (II) and Cd (II) ions was responsible for shifted peaks observed from 1045 cm^{-1} to 1043 and 1041 cm^{-1} for loaded with Pb (II) and Cd (II) respectively [25and 26].

XRD

XRD has been widely used to help characterize vital sorbents and verify the biological absorption mechanisms of heavy metals [27]. Results of XRD patterns of *S. oneidensis* (Fig. 2) Shows three sharp peaks at 44, 64.5, and 77.5^{o} corresponding to (200), (311), and (620) respectively, which indicating the sample is crystalline in nature. Slightly less changes in the severity of the three peaks of the unfilled biosorbents after contact to Lead (II) and Cadmium (II), this may explain the idleness manner. This findingis in consistence with these results indicated in the literature [28; 29 and 30]. By further comparing Fig. (2) The XRD diffraction peaks become lower,this which can be resulted from the fixing of Pb (II) and Cd (II) on the surface of *S. oneidensis* [31].

Transmission electron microscope

From figure (1) explain the considerable changes in the cell surface morphology later exposure to the metal ions. Results indicated bounded the metals within the cell membrane of bacterial cells,within some accumulates inside the cytoplasm of the cell. These results agree with Merroun *et al*. [32] they were stated that examining the electron microscope of three bacterial species *Acidithiobacillus ferrooxidans* exposed to uranium had proven uranium

placement in all of them cellular. Mullen *et al*. [33] indicated through the result of the TEM, presence of Ag^{+2} as the presence of particles at or near the gram positive and negative cell membranes. Panak *et al*. [34] they stated that the components of cell walls containing phosphates such as polysaccharides, teichoic and teichuronic acids or phospholipid layers can maintain the cellular form of bacteria, while the active groups are responsible for capturing external pollutants such as uranium. Sinha and Mukherjee, [35] showed that, amassed Cd^{+2} by *P. aeruginosa* as Electron-dense granules in cellular juices and toward the cell's shell. Also, El-Helow *et al*. [36] and Soltan *et al*. [37] stated that the surface of bacterial cells exposed to cadmium chloride becomes coarse and dilated as an adaptation by it for such exposure. The results of the current work also mark changes in the cells shape and size, which contact with heavy metals and that may be resulted in the change in the cell surface as a result of these contacts. These results are in accordance with Singh *et al*. [38] where they indicated morphological changes of the cell surface in Cryptococcus sp can be observed as a retracted cell wall and deformed in the existence of Cadmium and reductions in the existence of Led and Zinc. Numerous reasons contribute to these changes in the bacterial surface as a result of exposure to such pollutants. Excretion of polymeric substances by *Desulfovibrio desulfuricans* through absorption of Zn and Cu may be responsible in the modifying the cell surface morphology [39]. Similarly, El-Meleigy *et al*. [40] reported differ changes occur within the *B. firmus* and *B. subtilis* after Co^{+2} precipitation,these changes included the dark and dense cytoplasm and the cell wall thickness.

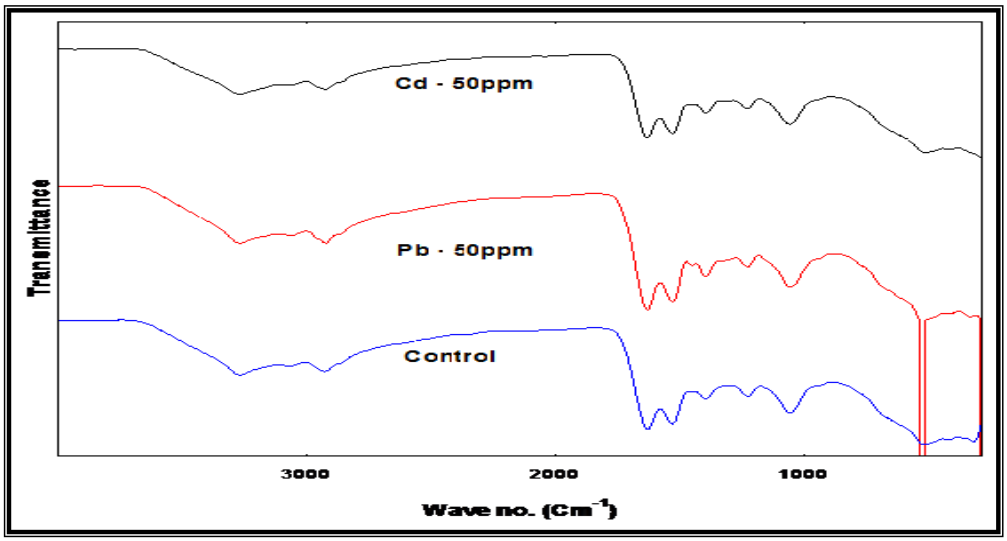

Figure 1: The FTIR Spectra of *S. oneidensis* unloaded and loaded with Lead (II), and Cadmium (II) ions.

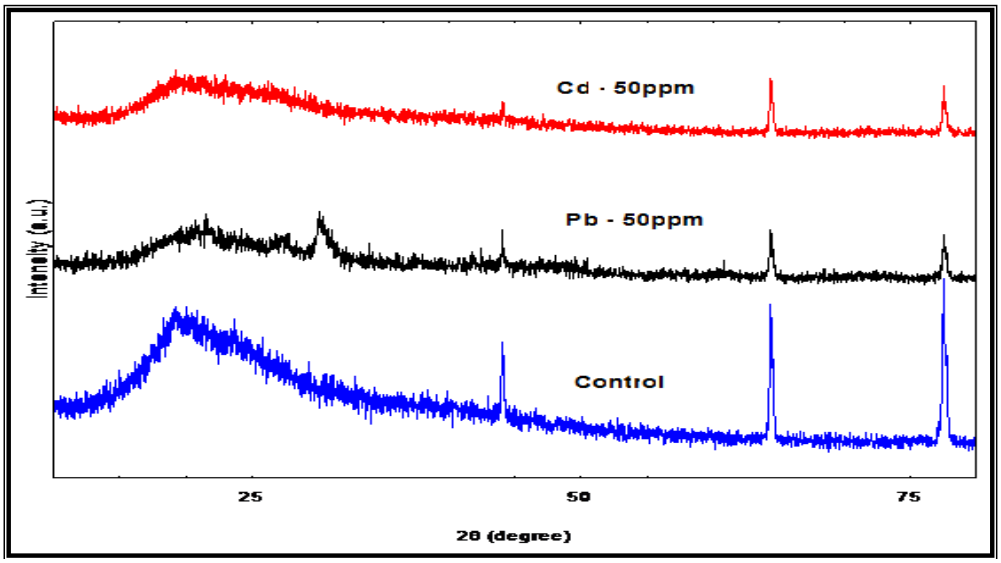

Figure 2: The analysis of biosorbent before and after loading with Pb and Cd.

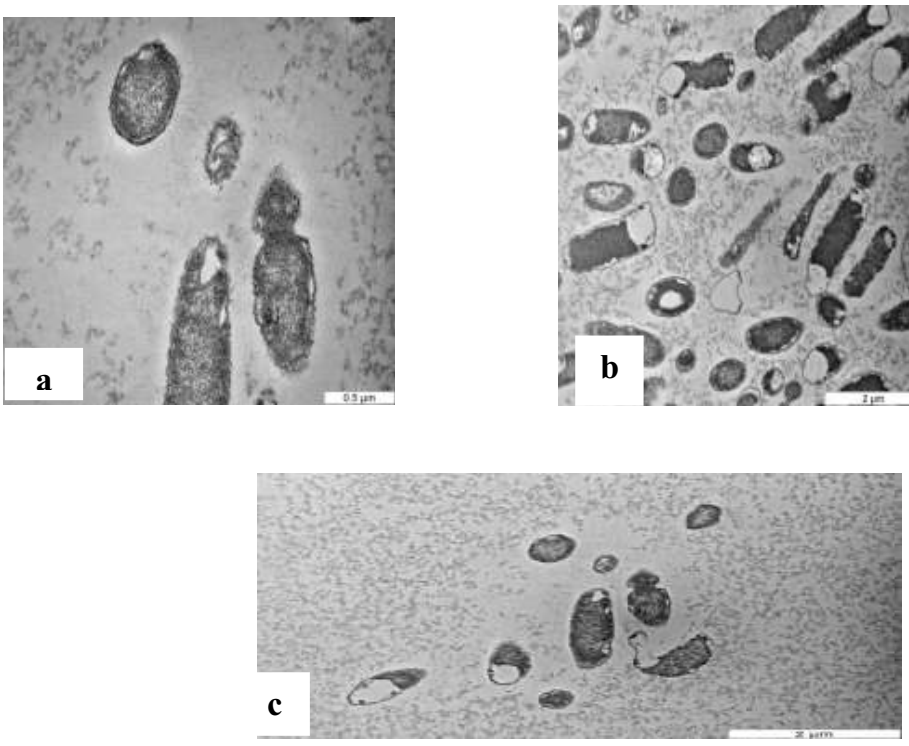

Figure 3: Transmission electron micrographs of *S. oneidensis*, a: control, b: contact to Cd, c: contact to Pb(Scale of bar 0.5µ and 2 µ).

Conclusion

S. oneidensis isolated from the soil polluted with Lead and Cadmium in the Basra city south of Iraq, showed higher ability to tolerate both these metals with the possibility of using in the bioremediation of heavy metals by two process, bioaccumulation and absorption.

Acknowledgment

The authors would like to express their gratitude to the biotechnology department, University of Putra, Malaysia and Marine Science Center, University of Basra.

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