Agriways 7 (2) : 107-109 December, 2019

ISSN: 2321-8614 (Print)
ISSN: 2454-2318 (Online)

Research Article

SPECTROSCOPIC AND SCANNING ELECTRON MICROSCOPIC STUDIES OF BIOSORPTION OF HEAVY METALS BY BACILLUS SPECIES

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Received: 19 Sept 2019/Accepted: 15 Nov 2019

Abstract Biosorption through bacterial biomass can be very effective technique for the treatment of heavy metal polluted waste water resulting from humans and industrial activities. In this study, biosorption of Cr (VI) was determined by Fourier transform infrared (FT-IR) and scanning electron microscopy (SEM). FT-IR analysis revealed that the possible interactions between metal ions and functional groups, such as amino, hydroxyl, carboxyl, and carbonyl present on cell surface of Bacillus sp. SEM analysis revealed that the morphological changes on cell surface of the Bacillus sp. after the exposure of Cr (VI).

Keywords: Biosorption, FTIR, SEM, Hexavalent Chromium

Introduction

Chromium is one of the world's most critical and extensive used materials among the metal used in chemical industries. The most common forms of chromium are Cr (II), Cr (III) and Cr (VI). Cr (VI) is the most toxic form of chromium that is mostly found at contaminated site. Wong and Trevors, (1988) analyzed that Cr (VI) is very toxic and mutagenic for humans and other organisms. Ajmal Rehman, (2001) stated that Cr (VI) in humans causes annoyance, corrosion of the skin, problems in respiratory tract and lung carcinoma. Microorganisms are able to tolerate high concentration of heavy metals due to developing several internal mechanisms. Tsezos, (2001) and Volesky, (2001) studied that biomass of algae, fungi and bacteria have been known to readily adsorb or accumulate most of metal ions. Biosorption of heavy metal ions is depends on affinity between metallic species or its ionic forms and the binding sites on the microbial cell membrane, cell wall and capsules (Raras, 1995).

These processes are important to industries because the removal of potentially toxic heavy metals and radionuclide from industrial wastewater by microbial biomass. It can lead to detoxification and also to recovery of valuable elements after proper treatment of the wastewater (Brierley et *al*., 1985). The interactions of metal ions and functional groups present on cell surface of the microbial biomass were confirmed by FT-IR and SEM analysis. FT-IR is one of the fast and prevailing tools to obtain information on polymer structure present in microbial biomass, because every chemical compound has its own distinct contribution to the absorbance/transmittance spectrum. Infrared spectroscopic investigation was performed in order to give a qualitative and preliminary characterization of the main functional chemical groups present in bacterial biomass which are responsible for heavy metal adsorption. The surface morphology of microbes could be studied under SEM (Kuo, 2007).

Materials and methods

2.1 Fourier Transform Infrared Spectroscopic (FT-IR) Analysis

Cr (VI) solution (100 ml of 100 mg/l) was added to 250 ml conical flask containing 500 mg *Bacillus* sp. biomass. Flasks were shaken at 240 rpm on rotary shaker for 2 hrs. After shaking, suspension was transferred to centrifuge tube. Suspension centrifuged for 15 minutes at 9000 rpm after centrifugation supernatant was discarded and pellets were dried at 60^0C. Dried biomass *Bacillus* sp. treated with Cr (VI) and untreated was powdered by pestle motor. *Bacillus* sp. biosorbent [Cr (VI) loaded and unloaded] were dried overnight due to removal of water because water retained in biosorbent could interfere with observation of hydroxyl groups on the biosorbent surface. Sample disks were made with 5mg of *Bacillus* sp. nonliving biomass encapsulated in 150mg of KBr. After that, sample discs were scanned into transmission mode through a wavelength range between 400 to 4000 cm^{-1} using FT-IR spectrophotometer. FT-IR spectra of Cr (VI) loaded and unloaded bacteria were recorded.

2.2 Scanning Electron Microscopy (SEM) Analysis

Scanning Electron Microscopy was used to identify morphology of the *Bacillus* sp. cell surface before and after adsorption. The surface structure of the untreated *Bacillus* sp. as well as that treated with Cr (VI) at a concentration of 30 mg/l was analyzed by SEM. The pellet from the experiment was washed thoroughly with sterilized triple distilled water (TDW), immersed in glutaraldehyde (2.5% Fluka) for 2 hours at room temperature and washed thoroughly with sterilized TDW. The pellet was then subjected to osmium tetraoxide staining (2% v/v, Fluka) for 1 hour and washed thoroughly with sterilized TDW. Next, the pellet was dehydrated by transferring it into a series of 25, 50, 70, 90 and 100% (v/v) of ethanol (Fluka) for 5 min. The final dehydration in 100% ethanol was carried out for 10 min. The dehydrated pellet was then dried overnight in an oven and mounted on a glass slide 120 stab with a double-stick carbon tab followed by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs.

Results and Discussion

3.1 FT-IR Analysis

The FT-IR spectra of non-metal and metal-loaded biosorbents, confirmed the presence of functional groups present in cell surface. These functional groups are responsible for the sorption process. FT-IR spectra taken at the wavelength between 400–4000 cm^{-1}. From the table 1 it is concluded that the binding of metal with respective groups of the targeted biological sample fall on 500 to 1500 cm^{-1} region of FT-IR spectrum and this is clearly depicted in fig. 1. FT-IR spectra of *Bacillus* sp. had intense absorption bands around 3200-3500 cm^{-1} which represent the stretching vibrations of amino groups, clearly reveals the vibration peaks (Table 1). The peak at 661, 845, 1180, 1060 and 1480 cm^{-1} are not present in with-metal which indicate the binding of Cr(VI) in the sample (Fig. 1). The peak is obtained at 845 and 1650 cm^{-1} in the unloaded *Bacillus* sp. while it is shifted to 864 and 1930 cm^{-1} in loaded *Bacillus* sp. Naik *et al.* (2012) also observed broad absorption change between 3000 and 3700 which was depicted bending vibration on O–H, N–H and O–H amino groups of proteins and water representing hydration of heavy metal. Changes at 2,368 cm^{-1} showed the stretching of C=C groups. The FTIR spectra show little differences in the nature of functional groups and the overall charging behaviour between control, and Cu (II) treated cells of *E. cloacae* AB6. As expected, the main functional groups are associated with proteins, phospholipids and polysaccharides (Rheinheimer, 2004). Pandiyan and Mahendradas (2011) also investigated comparable result using *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* for nickel removal.

Table 1: Assignment of infrared absorption bands

Wave numbers (cm^{-1})	Intensity shape	Assignment
3600-3750	Sharp	O-H streching
3400-3550	Sharp	O-H streching
3100-3500	Strong broad	N-H streching
2500-3400	Weak broad	O-H streching
2700-2950	Variable	C-H streching
1400-1660	Variable	N-H streching
1280-1430	Variable	C-H streching
1160-1420	Variable	O-H streching
900-1350	Variable	C-N streching
900-1380	Variable	C-O streching
800-880	Medium strong	N-H and C-H rocking

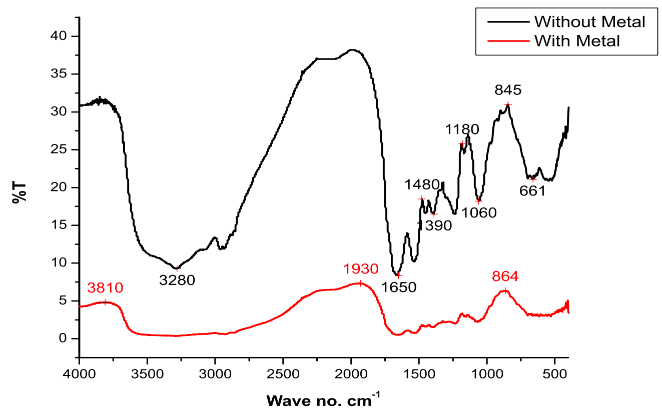
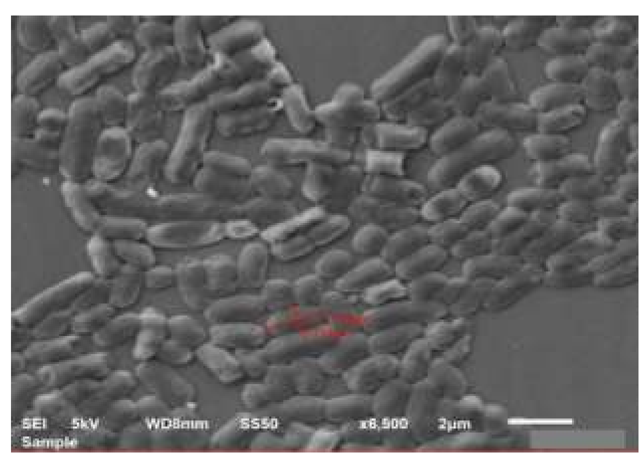


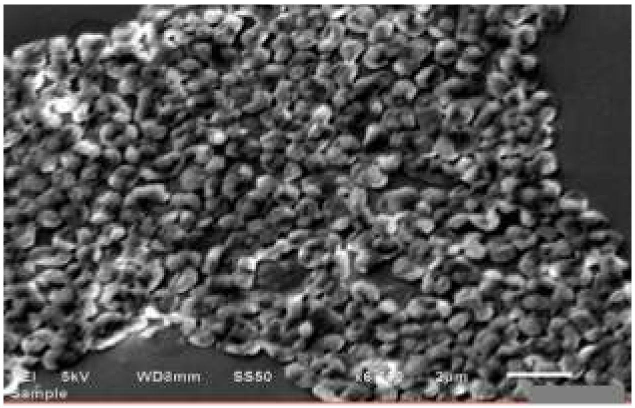
Fig. 1: FT-IR Spectra of *Bacillus* sp. in Cr (VI), (a) with and (b) without metal loaded

3.2 Scanning Electron Microscopy

The adsorption of Cr (VI) by *Bacillus* sp. was analyzed by scanning electron microscopy to understand its surface morphology with the magnification of 65,00x. SEM micrograph of unloaded and loaded with Cr (VI) *Bacillus* sp. is shown in fig. 2 (a- before sorption) and (b-



(a) Unloaded



(b) Loaded

Fig. 2: SEM micrograph of
(a) before and (b) after Cr (VI) uptake by *Bacillus* sp.

after sorption). Cell wall of bacteria had undergone significant physical disintegration after sorption. Before sorption of Cr (VI) bacterial cell was smooth, elongated and rod-shaped and after the sorption the cells were no longer smooth, elongated and rod-shaped. After sorption, significant change occurred in morphology, i.e. small and round-shape with uneven edges on cell wall of *Bacillus* sp. This indicates that metal particles adhere on the surface of the *Bacillus* sp. cell wall. Change in smoothness, shape, size and arrangement of bacterial cells occurs due to the adsorption of metal on bacterial cell wall. Morphology of *B. subtilis* through SEM analysis before and after uptake of metal ion, the cell surface of the bacteria becomes disintegrated and rough after metal uptakes (Sethuraman and Kumar, 2011). Ajmal Ahamed, *et al.,* (2013) also studied the morphology of biosorbent before and after sorption of metal ions by SEM. After uptake of metal ions on cell surface of biosorbent, the morphology of the bacteria had undergone significant substantial disintegration. Murthy *et al.* (2012) studied the morphology of bacterium cell surface by SEM and energy dispersive X-ray spectroscopy. He found that after uptake of Pb^{++} cell surface of *Bacillus cereus* has been disintegrated.

Conclusion

It is concluded that biomass of bacteria could be excellent, economic and eco-friendly for the removal of toxic and hazardous metal ions present in industrial wastewater. There are so many techniques exist for the characterization of biosorbents, a combination of scanning electron microscopy with EDX and FTIR is commonly required to obtain a complete description of the structure and surface functional groups. The mechanisms of adsorption could be the joint act of electrostatic interaction, complexation and ion-exchange between functional groups present on bacterial cell surface and metal ions. Use of *Bacillus* sp. biomass could be an effective, economic, eco-friendly and safe alternative for wastewater treatment generated from various industries.

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