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HEXAVALENT CHROMIUM DETOXIFICATION BY ALKALIPHILIC BACTERIA ISOLATED FROM TANNERY WASTE WATER IN LEBANON

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ABSTRACT

The present study deals with the application of Cr (VI)-resistant bacterial species in the bioaccumulation of Cr(VI) in alkaline growth conditions (pH 10). These bacterial species were isolated from wastewater samples collected from the outlet point of two leather tanneries in Saida, South Lebanon. The alkaliphilic isolates were investigated for Cr (VI) tolerance ability. Two bacterial isolates (A1 and A2) showed a maximum tolerance concentration (MTC) of 250 ± 0.0 and 50 ±0.0 mg L^{-1} of Cr(VI), respectively. The bacterial isolate (A1) with highest MTC to Cr (VI) was identified phylogenetically as *Bacillus cohnii* A1 (MK 478809). Mechanism of Cr (VI) accumulation by the living cells of *B. cohnii* A1 was investigated using Fourier Transform Infrared spectroscopy and Transmission Electron Microscopy. The optimization study revealed that the highest Cr(VI) removal from an aqueous solution of 10 mL volume was attained with a bacterial biomass of 0.1 g at pH 9. The bioaccumulation capacity and the kinetics of the process were determined. The effectiveness of the investigated Alkaliphilic *B. cohnii* A1 suggests its application in the *in situ* treatment of alkaline Cr (VI) polluted wastewater.

KEY WORDS: Hexavalent chromium, Alkaliphilic bacteria, *Bacillus cohnii*, Bioaccumulation

INTRODUCTION

Chromium is the 17th abundant element in earth (Avudainayagamas *et al.*, 2003). It exists in several oxidation states from $^{+}2$ to $^{+}6$ (Smith *et al.*, 2002) where the trivalent (Cr (III)) and the hexavalent (Cr (VI)) chromium are found to be the most prevalent and stable forms present in nature. Those species (Cr (III) and Cr (VI)) have different properties in terms of mobility, toxicity and bioavailability. The hexavalent chromium is a mobilized species due its high solubility in water, toxic and bioavailable whereas the reduced form of the metal ion (Cr (III)) is relatively immobile, less toxic and more stable (Viamajala *et al.*, 2004). The metal has been known for its extensive uses in industries particularly leather tanning industry, the cause that led to chromium polluted soil and ground water in which large quantities of chromium were found in the effluents of leather tanneries improperly discharged into the environment thereby posing serious threats to living biota including human (Gutterres *et al.*, 2015; Ashraf *et al.*, 2018).

As far as chromium effect on human health is concerned, Cr(VI)can result in serious health issues. This hazard is known for its carcinogenicity, mutagenicity and teratogenicity in humans (Flores and Perez, 1999), whereas Cr(III) is an important element needed in trace amounts for glucose, lipid and amino-acid metabolisms (Viamajala *et al.*, 2004). Due to the different toxicological characteristics of both species, acceptable concentration limit of 1 µg L^{-1} and 8 µg L^{-1} for Cr(VI) and Cr(III) in water are recommended (Chandra *et al.*, 1997). Moreover, a standard value of MCL (maximum contaminant level) of 0.1 mg L^{-1} for total chromium in drinking

water was set by the US-Environmental Protection Agency (US-EPA) in 2018.

Great efforts have been paid towards the development of effective technologies to remove hexavalent chromium from water. Technologies such as precipitation (Patterson, 1977), ion exchange (Tiravanti *et al.*, 1997) and adsorption (Orhan and Buyukgangor, 1993; Dahbi *et al.*, 1999) have been extensively used to remove the toxic metal ion from the aqueous system. However, these applications have one or more drawbacks in terms of cost, selectivity, high dependency on the concentration of the metal ion in addition to the fact that they are not eco-friendly (Katiyar and Katiyar, 1997). On the other hand, biological methods such as bioaccumulation, biosorption and bioreduction have been recognized as a promising tool for the sustainable treatment of Cr(VI) contaminated sites (Jeyasingh and Philip, 2005; Chai *et al.*, 2009; Gonzalez *et al.*, 2014; Sanjay *et al.*, 2018). Unlike the conventional physicochemical methods, the deployment of microbes in the remediation of Cr(VI) particularly from wastewater is a desirable approach due to the following advantages: (1) cost-effective solution, (2) environmentally friendly and (3) highly effective method (Jiang *et al.*, 2017).

Previous research have shown that different bacterial species such as *Bacillus* sp., *Microbacterium* sp., *Arthrobacter* sp., *Serratia*sp. and *Providencia* sp. are capable of reducing Cr(VI) to Cr (III) intracellularly *via* enzymatic processes (Sedlak and Chan, 1997; Kim *et al.*, 2001) or/and transform it to a much reduced toxic Cr(III) forms such as, chromium fluoride phosphate, calcium chromium oxides and other organo-Cr(III) precipitants (Srivastava and Thakur, 2012). In fact, chromate reduction by bacteria has been mostly conducted under acidic or nearly neutral pH conditions whereas very few studies on bacterial detoxification of Cr(VI) under alkaline conditions have been reported in the literature (Ye *et al.*, 2004; Stewart *et al.*, 2007; Van Engelen *et al.*, 2008; Abhay *et al.*, 2016). Thus, the present study elucidates the detoxification of Cr(VI) under alkaline conditions using alkali-tolerant chromium resistant bacteria isolated from tannery waste water. The reason that led us to the choice of such bacterial species is their adaption to high saline and extreme alkaline environments similar to those present in tanneries effluents (Zavarzin *et al.*, 1999). Phylogenetic characterization of the Cr (VI)-resistant bacterium is reported. Mechanism of Cr(VI) detoxification along with the optimization of the process is emphasized.

MATERIALS AND METHODS

Sampling and sample analysis of tannery effluents

Five waste water samples were collected from the outlet point of two leather tanneries in Saida, South Lebanon. Water samples were transferred to sterile plastic containers, stored at 4 ± 2 °C prior to analysis and bacterial isolation. Electrical conductivity and pH of water samples were measured using a conductivity meter (Mi 170 Bench Meter) and a pH meter (Ohaus starter 3100). Chromium content of the water samples was determined using an Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3000 series) after they have been acid digested.

Isolation and culture conditions of bacterial isolates

Isolation of alkaliphilic bacteria from the collected waste water samples was performed using enrichment growth medium, Horikoshi I broth (pH 10.0) consisting of (g L^{-1}): glucose 10, yeast extract 5, peptone 5, K_2HPO_4 1, $MgSO_4$ $7H_2O$ 0.2 and Na_2CO_3 10. For medium solidification, agar (20 g) was added to make the Horikoshi I agar (Horikoshi, 1999). Aliquots of the liquid medium (50 mL) were dispensed in sterile Erlenmeyer flasks after which they were inoculated with the wastewater sample (1 mL) and placed in a shaking incubator (ZHWY-2102C) with 150 rpm at 35 ± 2 °C for 48 hours. Following the incubation period, 0.1 mL from each flask was transferred to Horikoshi I agar plate and incubated at 35 ± 2 °C for 48 hours. Further purification of the bacterial colonies was made *via* streaking method using the same growth medium (Horikoshi I agar) after which they were then examined microscopically with Gram staining.

Determination of hexavalent chromium resistance profile of isolated alkaliphilic bacteria

The bacterial isolates were screened for their ability to tolerate different concentration levels of chromium solution (Cr(VI)) using broth micro dilution method (CLSI 2012). Two-fold serial dilutions of Cr(VI) (nitrate salts) (starting concentration 1000 mgL^{-1}) were introduced to 10 wells of one row. The prepared bacterial suspensions adjusted to 0.5 Mcfarland (1.5×10^8 CFU/mL) were diluted in sterile Horikoshi I broth to reach 1×10^6 CFU/mL, then 50 µL of each dilution was added into each well (column 1-10) of one row

resulting in a final desired inoculum of 5×10^5 CFU/mL (Balouiri *et al.*, 2016). The plates were then incubated for 16 to 20 hours at 35 ± 2 ^{0}C. The maximum tolerance concentration (MTC) of Cr (VI) for a particular bacterial isolate was determined as the maximum concentration of the metal ion in which bacterial growth was positive. Bacterial isolate(s) that showed high tolerance to Cr(VI) was/were selected for further experiments.

Phylogenetic characterization of the selected bacterial isolate

Phylogenetic characterization of the selected bacterial isolate with highest Cr(VI) tolerance was carried out using 16S rRNA sequence analysis. The total genomic DNA of a single colony was extracted and purified according to the method described by Ausubel *et al.* (2003). The bacterial 16S rDNA was amplified from the total genomic DNA using universal eubacteria specific primers, designated to amplify 1500 bp fragment of the 16S rDNA regions. The forward primer was: 8F (AGA GTT TGA TCC TGG CTC AG) and the reverse primer was: U1492R (GGT TAC CTT GTT ACG ACT T), which yielded a product of approximately 1500 bp. The PCR product was purified using Gene JET™ PCR Purification Kit (Thermo K0701). The purified product was sequenced in GATC Biotech (Germany) usingABI 3730xl DNA sequencer. The PCR product was sequenced using the same PCR primers. The sequences obtained were aligned with known 16S rDNA sequences in the Genbank database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/), and percent homology scores were generated to identify the bacterial isolates. Phylogenetic tree was constructed with MEGA version 3 (Kumar *et al.*, 2004).

Conductivity and FT-IR measurements

Cr(VI)-resistant isolate was inoculated in Horikoshi I broth culture medium on a shaking incubator at 30 ± 2 ^{o}C. The culture was harvested in its exponential phase (approximately 18 hours), centrifuged at 6000 rpm for 15 minutes and washed three times with deionized distilled water. Bacterial pellet (0.1 g) was transferred to 10 mL volume of 0.1 mmol L^{-1} Cr(VI) (nitrate salts) solution prepared in deionized distilled water with pH 10. The mixture (bacterial cells and Cr(VI)) was agitated for 5 minutes then incubated overnight at 30 ± 2 ^{o}C. During the incubation period, conductivity measurements of the Cr(VI) solution were recorded at different time intervals (0, 60, 120, 240, 1080 and 1440 minutes) using conductivity meter (Mi 170 Bench Meter).

Following the incubation period, the bacterial pellets were collected and dried prior to FT-IR measurement. The conformational changes of the functional groups of the investigated bacterial isolate upon its interactions with Cr(VI) was detected *via* a Fourier Transform Infrared (FT-IR) spectrometer (Thermo Scientific Nicolet iS5 FT-IR) using the procedure described by Bhattacharya and Gupta (2013). Spectra of the dried biomass of untreated and Cr(VI) treated bacterial cells were recordedin the region of 400-4000 cm^{-1} with 32 scans.

Transmission electron microscopy imaging

The localization of Cr(VI) within the bacterial cells was identified using Transmission Electron Microscope (TEM) (JEM-1400 Plus), at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt. Bacterial cells were fixed using universal electron microscope fixative. A series of dehydration steps were made to the pellets using ethyl alcohol and propylene oxide. The pellets were then embedded in labeled beam capsules and polymerized. Thin sections were obtained using LKB 2209-180 ultra-microtome, stained with a saturated solution of uranyl acetate for 30 minutes and lead acetate for 2 minutes (McDowell and Trump, 1976). The same procedure was applied to control bacterial cells (Cr(VI)-unloaded cells). The magnification used for the investigated samples was 15000x and accelerating voltage was 80 kV.

Hexavalent chromium uptake experiments by the investigated bacterial isolate

The identified bacterial isolate was investigated for its ability to remove Cr(VI) (nitrate salt) from aqueous solutions using batch procedure. Parameters affecting the metal ion uptake process such as, biomass dose, pH of the solutions, Cr(VI) initial concentration and kinetics were investigated.

Biomass dose

To determine the effect of bacterial biomass on the extraction of hexavalent chromium, overnight cultures of the bacterial isolate were centrifuged at 6000 rpm for 15 minutes to obtain bacterial pellets with the following masses: 0.05, 0.1, 0.25, 0.5 and 0.75 grams. Cr(VI) solution (10 mL, 0.1 mmolL^{-1}) was prepared in deionized distilled water then

added to the various amount of the bacterial pellets in polypropylene tubes. The tubes were agitated for 5 minutes and incubated overnight to equilibrate at 30 ± 2 ^{0}C. The samples were filtered using 0.45 µm millipore membrane filters and filtrates were analyzed for chromium concentration. The tested supernatants were diluted by deionized distilled water and no further treatments were applied before being analyzed by Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3000 series). Calibration curves were completed before the samples were run where double deionized water (DDW) was the blank experiment and analysis was carried out in triplicate. Chromium extraction percentages (% E) were calculated according to the following equation:

$$\% (E) = (C_i - C_f)/C_i \times 100 \quad \text{.. Eq. (1)}$$

C_i and C_f are initial and equilibrium Cr(VI) concentrations (mmol L^{-1}) in water.

Effect of solution pH on Cr(VI) removal

The pH effect of chromium salt solutions in aqueous medium on the removal ability of the investigated bacterial isolate was examined in the 6-10 pH range. Chromium solution (10 mL, 0.1 mmol L^{-}1) was placed in a polypropylene tube containing an optimum mass of the bacterial isolate. The pH of the solutions was adjusted by adding 0.1 mol L^{-1} H_2SO_4or 0.1 mol L^{-1}NaOH solution. All samples were shaken for 5 minutes and then incubated overnight at 30 ± 2^{0}C. Following the incubation period, samples were taken and analyzed for the remaining Cr(VI) concentration using the procedure described in the previous section. The extraction percentage (% E) was calculated using Eq. (1).

Uptake capacity of Cr(VI)

The capacity of the bacterial isolate to uptake Cr(VI) from aqueous medium was investigated using batch technique. Thus, known volume (10 mL) of different concentrations of Cr(VI) (0.1-5 mmolL^{-1}) with adjusted optimal pH value were added to an optimal mass of the bacterial isolate. Mixtures were agitated for 5 minutes then left overnight to equilibrate at 30 ± 2 ^{o}C. The uptake capacity, q_{eq} (mmol.g^{-1}) amount of Cr(VI) taken up per unit of mass of bacterial pellet was calculated using the following Eq:

$$q_{eq} = \frac{(C_i - C_{eq}) \times v}{m} \qquad \text{Eq. (2)}$$

In Eq. (2), V denotes the sample volume (mL), c_i and c_{eq} are the initial and equilibrium Cr(VI) concentrations (mmolL^{-1}) respectively, and m is the amount of the bacterial pellet used.

Kinetics of the Cr (VI) removal process

The kinetics of the Cr (VI) removal process was determined using the optimal experimental conditions but at different time intervals (5, 20, 30, 60, 120, and 1440 min), q_{eq} (mmol g^{-1}) and it was calculated using Eq. (2), half-life values for chromium removal were determined from the plots of the bacterial isolates uptake capacity (q_{eq}) *vs* the time in minutes.

RESULTS AND DISCUSSION

Analysis of tannery effluents

The electrical conductivity (EC), pH and chromium content values of the five collected samples from the tannery effluents are shown in Table 1. All the checked parameters were found in higher levels than the standard permissible limit (ISW-BDS-ECR 1997; ISI-2000; NEQS 2000). Therefore, further investigations have been carried out to isolate and identify the microbial species that are adapted to such harsh environmental conditions. These microorganisms have the advantage of being useful in the treatment of chromium contaminated water. Findings and discussions are given in the following section.

Table 1. Physicochemical properties of tannery effluents

Parameters	S1	S2	S3	S4	S5	Standard Permissible Limits		
						ISI (2000)	NEQS (2000)	ISW-BDS-ECR (1997)
EC (mS/cm)	39.40	22.11	84.80	67.10	53.20	0.85	0.288	-
pH	7.72	8.63	12.30	12.17	10.20	6-9	6-9	6-9
Chromium (mg L^{-1})	53.30	41.30	75.40	70.60	68.20	1.0	1.0	0.5-1.0

ISI-2000=Indian Standard Institute–2000, NEQS (2000) = National Environmental Quality Standards-2000, ISW-BDS-ECR =Inland Surface Water-Bangladesh Standard.

Chromium tolerance of the bacterial isolates

The alkaliphilic bacterial strains (n=10) isolated from tannery effluents, were investigated for their resistance to hexavalent chromium *via* qualitative assay. Two bacterial isolates were selected as they showed obvious visible growth in the culture medium supplemented with $Cr(NO_3)_6$. Consequently, these two isolates (A1 and A2) were checked for their highest tolerance concentration to Cr(VI) by broth micro dilution assay. The bacterial isolates A1 and A2 were found to tolerate up to 250 ± 0.0 and 50 ±0.0 mg L^{-1} Cr(VI), respectively (Fig. 1). Previous studies have reported a chromium tolerance range of 100 to 4000 mg$L^{-1}$by microorganisms (Thacker *et al.*, 2007; Zhu *et al.*, 2008). Hence, A1 was then selected for further investigations as it showed resistance to high Cr(VI) concentrations.

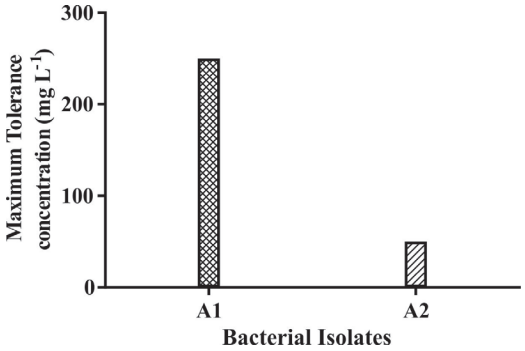

Fig. 1. Maximum tolerance concentration (mg L^{-1}) of isolated alkaliphilic bacteria to Cr (VI)

Morphological and phylogenetic characterization of the Cr(VI) resistant bacterium

The microscopic observation of Cr (VI) resistant bacterium (A1) displayed rod shaped, gram positive bacterial cells. The 16S RNA gene sequence of the A1 revealed 99 % similarity with *Bacillus cohnii* NBRC 15565 (NR 113776.1). The phylogenetic tree presented in Fig. 2 illustrates its similarity with the other 16S rRNA sequences of relevant *Bacillus* species. Based on this phylogenetic characterization, the bacterial isolate was identified as a member of the genus *Bacillus* and named as *Bacillus cohnii* A1. The sequence of the 16S rRNA gene is available under GenBank accession number MK 478809. Spanka and Fritze (1993) coined out that *Bacillus cohnii* is an obligate alkaliphilic oval spore forming *Bacillus* species, flagellated, catalase and oxidase positive with incubation temperature range of 10-47 °C.

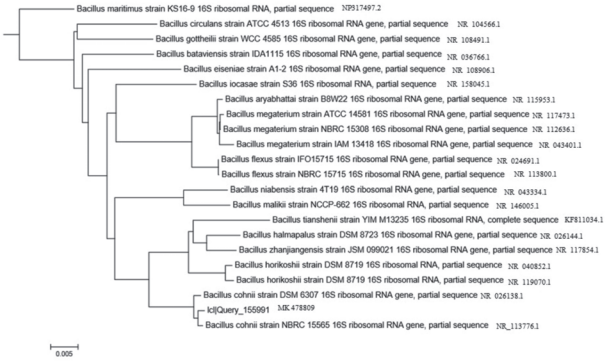

Fig. 2. Phylogram showing the position of *Bacillus cohnii* A1 (MK 478809) with the other members of *Bacillus* genus based on 16S rRNA gene sequence

Aqueous and solid phase analyses through conductivity and FTIR measurements

Conductivity of Cr(VI) aqueous solution (0.1 mmol L^{-1}) was traced prior and following to its interaction with the investigated Alkaliphilic *Bacillus cohnii* A1 at pH 10. Inspection of Fig. 3 reveals a decrease in the conductivity values within 18 hours where no significant changes were observed following that period. This suggests a gradual removal of the metal species by the bacterium. This finding was further supported with FT-IR analysis of the solid phase (bacterial pellets). Oves *et al.* (2013) demonstrated the importance of functional groups present in the active sites of bacterial cells in the removal of the metal ions from the environment. Therefore, FT-IR measurements were conducted for Cr(VI) treated and untreated *B. cohnii* A1 cells where spectra are presented in Fig. 4. The assignment of the bands was referred to the data available for the relevant functional groups. The infrared spectrum of Cr(VI)

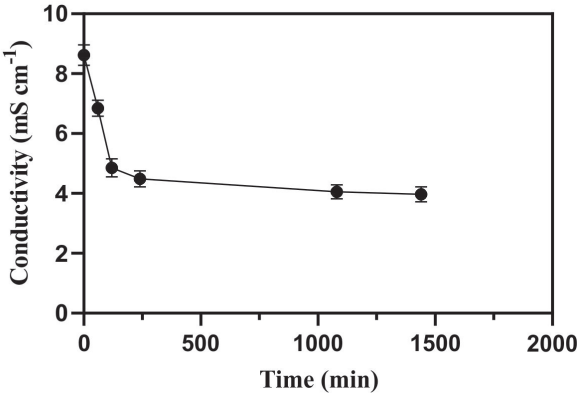

Fig. 3. Electrical conductivity curve of Cr(VI) (nitrate as counter ion) solution before and after being interacted with *Bacillus cohnii* A1 at 30 ± 2 °C (Error bar represents means±SD).

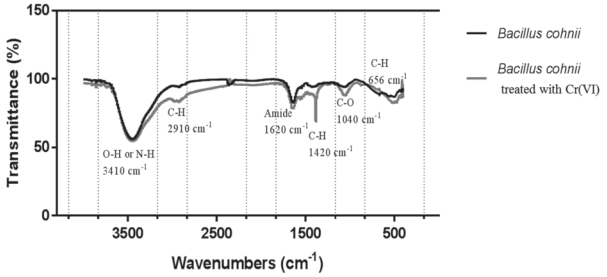

Fig. 4. FT-IR spectra of *Bacillus cohnii* A1cells and Cr(VI) containing *Bacillus cohnii* A1 cells displaying the most important bands of the solid samples.

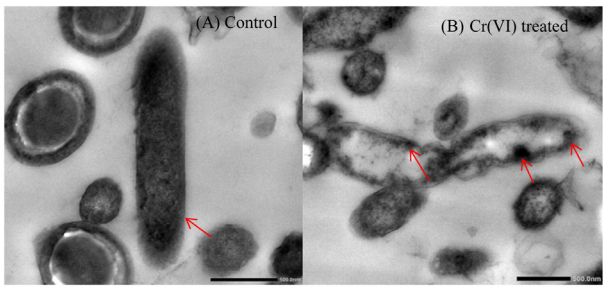

Fig. 5. Transmission electron micrographs of *Bacillus cohnii* A1, (A) control untreated cells and (B) cells treated with 0.1 mmol L^{-1}Cr (VI). Red arrows show the intracellular accumulation of Cr(VI) in the bacterial cells.

free cells presents a broad band at 3410 cm^{-1} which was assigned to O-H or N-H stretching vibrations. Another broad band appeared at 2910 cm^{-1} was corresponded to C-H stretching. The band at 1620 cm^{-1} was assigned to the C=O of amide groups. The weak band appeared at 1040 cm^{-1} was corresponded to C-O vibration which is a polysaccharides characteristic peak. As far as the IR spectrum of Cr(VI) treated cells is concerned, a slight red shift was observed at 1040 cm^{-1} and this could be due to the interaction of the metal species with the carboxyl functionality.

Gram positive bacteria bear specific anionic receptors in their cell wall structures such as teichuronic acids and peptidogly can which complex with metal ions, the fact that explains the main role of the microbial cell wall in metal ions interaction (Volesky, 1990). Another observed change in the IR spectrum of Cr(VI) treated A1 cells was the decrease in the transmittance of the peaks and this is referred to presence of metal ion in the cells that led to bond stretching at a lower degree. Our findings come in good agreement with previously reported studies (Norton *et al.*, 2004; Lodeiro *et al.*, 2006; Tunali *et al.*, 2006; Gabr *et al.*, 2008; Giotta *et al.*, 2011). However, it was not possible to pinpoint the removal mechanism of the metal species by the investigated bacterial isolate from the obtained findings as morphological observation was needed to localize Cr(VI) in the bacterial cell.

Localization of Cr(VI) within the investigated alkaliphilic bacterium as observed by transmission electron microscope

Transmission electron microscope was conducted to identify the Cr(VI) compartmentalization within the bacterial cell. Fig. 5A represents the electron micrograph of the control cells (cells not exposed to chromium solution) which showed a normal appearance with a smooth surface and no conspicuous electron dense areas. Clear and sharp cell wall and surface membrane were nicely seen. The modifications in the structure of the cell's compartments due to Cr(VI) treatment were recorded and are indicated in Fig. 5B. *Bacillus cohnii* A1 cells exposed to chromium demonstrate the deposition of the metal as black dots on the cell wall and the cell membrane. Moreover, the metal occupied in the cytoplasm is in localized precipitates (arrow). Microscopic observation of Cr precipitates in bacterial cells was reported by several studies that all suggested an intracellular reduction mechanism of Cr(VI) into Cr(III) and this demonstrates the effectiveness of such bacteria in the detoxification of Cr from the environment (Daulton *et al.*, 2007; Srivastava and Thakur, 2007; Dogan *et al.*, 2011; Naik *et al.*, 2011; Xu *et al.*, 2011).

Factors influencing Cr(VI) removal by *Bacillus cohnii* A1

Being an inexpensive and readily available source of biomass that can be obtained in large quantities makes it a good candidate for Cr(VI) removal. Moreover it was isolated from heavily contaminated water with chromium (75.40 mg L^{-1}) the property that makes it a good chromium scavenger.

Effect of bacterial biomass on hexavalent chromium removal process

Bacterial biomass dose is an important parameter affecting heavy metal removal from aqueous solutions. Results showed that Cr(VI) removal efficiency increased with the increase in the biomass of *B. cohnii* A1 from 0.05 g to 0.75 g although there was no significant difference between the metal ion removal at 0.25 g to 0.75g. The highest Cr(VI) removal from aqueous solution of 10 mL volume

was attained with an optimum biomass of 0.1 g (Fig. 6). This is due to the electrostatic interaction between the cells since as biomass concentration increase, the competition among the cells increase leading to interference between the binding sites. Thus high biomass concentration limits the access of the metal ions to the binding sites (Nuhoglu and Malkoc, 2005; Masoudzadeha *et al.*, 2011; Suriya *et al.*, 2013).

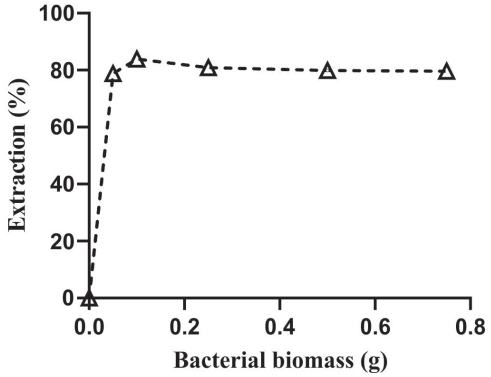

Fig. 6. Bacterial biomass effect on Cr(VI) extraction by *Bacillus cohnii* A1 (initial concentration 0.1 mmol.L^{-1}; pH 10) incubated at 30 ± 2 °C.

Effect of pH on hexavalent chromium removal process

In order to assess the role of pH in the extraction process, an experiment with varying pH values (6-10) was set to estimate their effect on removal ability of *B. cohnii* A1 biomass using a constant (0.1 mmol L^{-1}) concentration of Cr(VI). The extraction percentage increased as pH increased from 6 to 9 (Fig. 7) proving that the alkaline condition favors the extraction of Cr(VI) compared to neutral or acidic conditions. The optimum condition for the extraction of Cr(VI) was accomplished at pH 9 which coincide with the results obtained by Ma *et al.* (2007) and Mangaiyarkarasi *et al.* (2011).

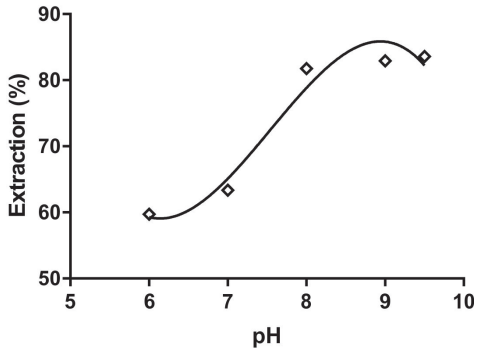

Fig. 7. Effect of pH on the extraction of Cr(VI) at initial concentration 0.1 mmol.L^{-1} by *Bacillus cohnii* A1 incubated at 30 ± 2 °C.

Hexavalent chromium bioaccumulation capacity by *Bacillus cohnii* A1

The removal capacity of *B. cohnii* A1 was studied to determine the maximum uptake of the metal ion per unit mass of bacterium. It was noticed that the removal capacity increased as the concentration of Cr(VI) increased until reaching a constant value (Fig. 8). This could be attributed to the binding of the Cr(VI) to all active sites in the bacterial surface area thus reaching a saturation level. Moreover, the no more Cr(VI) uptake by the investigated bacterial isolate was due to the fact that higher Cr(VI) concentrations led to cell toxicity during the 24 hr incubation period, this performance is in accordance with the findings of Shao *et al.* (2019). Notice that, *B. cohnii* A1 has consumed its need from the metal ion in the studied time interval.

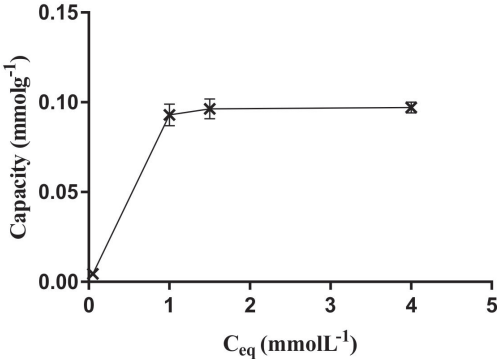

Fig. 8. Cr(VI) bioaccumulation capacity (mmol g^{-1}) of *Bacillus cohnii* A1 incubated at 30 ± 2 °C (Error bar represents means ± SD).

Kinetics of chromium removal process

The kinetics of the study showed that the optimum contact time for the uptake of Cr(VI) by *B. cohnii* A1 was at t(1/2)=40 minutes and this was due to an

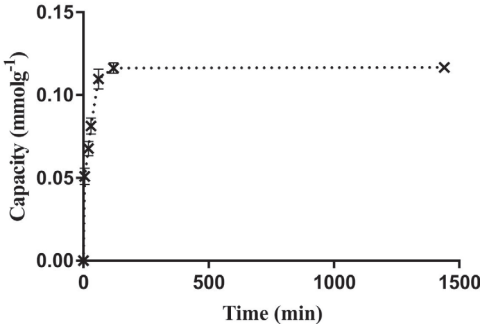

Fig. 9. Optimum time contact for the uptake of Cr(VI) by *Bacillus cohnii* A1 at 30 ± 2 °C incubation temperature. t(1/2)= 40 minutes; k= 0.017 s^{-1}(Error bar represents means±SD).

initial rapid metal uptake followed by slow uptake. The uptake of metal ions by microorganisms in batch systems has been shown to occur in two stages: an initial rapid stage (passive uptake), followed by much slower process (active uptake) (Abdel-Aty *et al.,* 2013; Dadrasnia *et al.,* 2015; Fadel *et al.,* 2017).

CONCLUSION

Bacterial isolate that showed a high resistance to hexavalent chromium was phylogenetically identified. Conductivity, FTIR and TEM results revealed the binding of the metal ion to the cell surface along with its precipitation in the cell cytosol which confirms its bioaccumulation inside the cell. Optimization of environmental parameters showed the highest removal of Cr(VI) can be achieved at 0.1 g biomass and pH range of 8.5-10. Given that, bacterial isolates are available in large quantities in addition to their low cost, it can be concluded that the removal of toxic pollutants from the environments using free living cells should be further explored, particularly with extremophiles since they owe the advantage of quick adaptation to any change in their environmental surroundings. The findings of the present study recommend the choice of alkaliphilic free living cells as an eco-friendly biosorbent for Cr (VI) remediation from wastewaters.

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