

ORIGINAL RESEARCH ARTICLE

Biosorption of Hexavalent Chromium Using *Bacillus Licheniformis* Isolated From Effluent SampleA. Ajmal Ahamed^{*1}, C. Ananda kumar² and S. Elamathy³¹Jamal Mohamed College, Trichy, Tamil Nadu, India²Sengunthar Arts and Science College, Tiruchengode, Tamil Nadu, India³PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

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ABSTRACT

Biosorption can be an effective technique for the treatment of heavy metal bearing waste water resulting from humans and industrial activities. In the present study, the bio sorption of heavy metals is using individual and mixed culture of attenuated bacteria (Gram positive and Gram negative) like *Bacillus subtilis* and *Pseudomonas aeruginosa* and parameters affecting the bio sorption of heavy metals; such as time, pH, biomass concentration and initial metal concentration have been investigated. The batch experiments have been carried out using individual and mixed bacterial culture and the bio sorption parameters were optimized using univariate procedures. In this present study, medium with different pH of was varied from 1 to 10 and the optimum condition for the bisorption was found to be pH - 2. Effect of temperature was investigated by maintain the conical flaks at 25°C, 27°C, 29°C, 31°C and 35°C. The maximum bio sorption was observed at 27°C with a biosorption efficiency of 54.5%. To study the effect of effect of biomass concentration, the experiments were carried out by varying biomass dosage from 1 mg to 10 mg⁻¹ in the biosorption experiments and it was found to be 1 mg⁻¹ gave a satisfactory results, even though a concentration of 9 mg⁻¹ gave the maximum results but as a fact of biomass usage, we selected 1 mg-1 for further optimization process. The amount of Cr(VI) adsorbed with cultures grown in glucose, fructose and sucrose with lower concentration of Cr(VI) (50mg/l) was about 4.3, 5.2 and 3.8 mg/g cell mass.

Key words: Biosorption, Heavy metals, *Bacillus subtilis* and *Pseudomonas aeruginosa*.**1. INTRODUCTION**

Industrialization has long been accepted as a hallmark of civilization. Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, moss, fungi or bacteria that have been killed while the biomass is pretreated by washing with acids and/or bases before final drying and granulation. Heavy metal ions are used in various industries due to their technological importance. Waste waters from these industries include metal ions having permanent toxic effect. Algae, fungi, yeast and bacteria remove heavy metals from waste waters through functional groups such as ketones, aldehydes, carboxyls on their cell walls. Arsenic, Chromium, Lead, Mercury, Nickel are some of the metal contaminants. Chromium (Cr) is one of the world's most strategic and critical materials having a wide range of uses in the

metals and chemical industries. Chromium is found in rocks, animals, plants, and soil. The most common forms of chromium are Cr (II), Cr (III), and Cr (VI). Steel is made from chromium (0). Cr (III) appears naturally in the environment in the form of chromium (II) and chromium (VI). The hexavalent chromium compounds (chromium VI) are industrial produced by the oxidation of chromium (II) compounds ^[1]. Cr (VI) is the form of chromium that is mostly found at contaminated sites. Hexavalent chromium is toxic and mutagenic to most organisms ^[2] and is known to cause irritation, corrosion of the skin and respiratory tract, and lung carcinoma in humans ^[1].

2. MATERIALS AND METHODS**Preparation of metal solutions**

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Stock solution (0.1g in 10ml) of Cr⁶⁺ was prepared by dissolving analytical grade of K₂Cr₂O₇, in double distilled water. Before mixing with the biosorbents, the stock solution was diluted to the required concentration

Preparation of bacterial biosorbents

For biosorption study, bacterial strain was cultivated aerobically in 500ml flask containing sterile nutrient broth on a rotary shaker 100 rpm at 37°C. Cells were harvested at the end of exponential phase. The cells were centrifuged at 9000 rpm for 10min and then washed twice with deionized water and finally dried in hot air oven at 60°C for 24 hours. The dried biomass was used for the biosorption experiments.

Analytical estimation of chromium

A 0.25% w/v solution of diphenylcarbazide was prepared in 50% acetone. 15ml each of the sample solutions, containing various concentrations of Cr (VI) were pipetted out into 25ml standard flasks. To this 2ml of 3M H₂SO₄ was added followed by 1ml of diphenylcarbazide and the total volume was made up to 25ml using deionized, double distilled water. Chromium concentration estimated by the intensity of the color complex formed was measured using a UV visible spectrophotometer. The absorbance was measured against a reagent blank at 540nm. A linear plot was obtained indicating adherence to the Beer Lambert's law in the concentration range studied.

Determination of biosorption percentage

The amount of metal ion adsorbed per gram of biomass and the sorption efficiency (%) were calculated according to the expression:

$$\% R = \frac{(C_o - C_e)}{C_o} \times 100$$

Where,

- % R = Percent removal of Chromium
- C_o = Initial metal concentrations (mg/ml)
- C_e = Final metal concentrations (mg/ml)

Biosorption studies were done using biomass as a function of various parameters such as

- a) pH
- b) Temperature
- c) Incubation time
- d) Biomass concentration
- e) Initial metal concentration
- f) Different Carbon sources

Effect of pH on biosorption

The metal biosorption monitored for pH range 1 to 10. NaOH and HCL were used as pH regulators. 1 mg/ml biomass was dispersed in 100

ml of the solution containing 1mg/ml of metal concentration. All flasks were maintained at different pH values ranging from 1 to 10 for about 24 hours. Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the metal ions using Diphenylcarbazide assay method^[3].

Effect of temperature on biosorption

Optimum biomass concentration with optimum pH was used to monitor the temperature effect on biosorption. Experiments were carried out at different temperatures 25°C, 27°C, 29°C, 31°C and 35°C, kept in shaker incubator for 24 hrs. Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazide assay method^[3].

Effect of incubation time on biosorption

In order to determine the incubation time on biosorption of Cr (VI) ions, the experiment conducted with 1 mg/ml biomass concentration dispersed in 100 ml of the solution containing 1mg/ml of metal concentration. An individual flask for each incubation time was maintained (6, 12, 18, 24, 30, 36, 42 and 48 hours). Samples were centrifuged after and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazide assay method^[4].

Effect of biomass concentration on biosorption

Biomass was centrifuged at 9000 rpm and different weights of the biomass ranging from 1 to 10mg/ml were dispersed in solutions containing the 1mg/ml metal concentration. The solutions were adjusted to the pH-2 and incubated at 27 °C and the supernatants were later centrifuged at 9000 rpm and the metal ion concentrations were determined using Diphenylcarbazide assay method^[4].

Effect of initial metal concentration on biosorption

To investigate the effect of initial metal concentration, biosorption experiments were conducted using conical flasks containing initial metal concentrations of 1 to 10 mg/ml working biomass concentration of 1mg ml⁻¹. Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazide assay method^[3].

Effect of carbon source on biosorption

Effect of different sugar source on biosorption experiments were conducted using conical flasks containing different sugars (1% conc.) glucose,

Sucrose, Lactose, Maltose, Mannitol and Fructose with an initial metal concentration of 1 mg ml⁻¹. The biomass concentration maintained was 1 mg/ml and the flasks were maintained at pH- 2 and temperature at 27°C for 24 hours. Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazide assay method [3].

Characterization of biosorbents

Fourier transform-infrared spectroscopy

FTIR is one of the rapid and powerful tools to obtain information on polymer structure, because every chemical compound in the sample makes its own distinct contribution to the absorbance/transmittance spectrum. Infrared analysis Infrared spectroscopic analysis for the samples under investigation was performed in order to give a qualitative and preliminary characterization of the main functional chemical groups present on the bacterial biomass which are responsible for heavy metal biosorption. A raw sample of bacterial biomass and biomass loaded with Cr⁶⁺ Were analyzed using an Infrared spectrophotometer (IR) Model 470 Shimadzu corporation adopting KBr disk technique.

Scanning electron analysis

The surface structure of biosorbent was analyzed by scanning electron microscopy (SEM) S-3400. Unloaded and metal-loaded biomass samples were mounted on aluminum stub sequenced by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs [5].

Desorption studies

The regeneration of the biosorbent may be crucially important for keeping the process cost down. The desorption process should yield the metal in a concentrated form, restore the biosorbent close to the original state for effective reuse with undiminished metal uptake and physical changes or damages to the biosorbent. The biomass was loaded with Cr(VI) was treated with 0.1 M NaOH at room temperature (27°C) with constant shaking for 2 hours [3].

Immobilization of biomass

A 2% (w/v) slurry of sodium alginate was prepared in hot (60 °C) distilled water. After cooling 0.1% of biomass was added and stirred. The alginate biomass slurry was then extruded into 50 mM CaCl₂.2H₂O for polymerization and bead formation. Biosorption studies were performed by dispersing 0.1 % beads in 50 ml of the solution containing 1mg/ml of metal

concentration. The flask was maintained at pH-2 at 27°C for 24 hours. Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazide assay method [3].

Antibiotic assay

To determine the antibiotic sensitivity of the bacterial isolates, antibiotic impregnated discs were placed on freshly prepared lawns of each isolate on nutrient agar plates. The plates were incubated at 37° C for 24 hours. The diameters of the inhibition zones were measured and observed for their resistant and sensitivity the selected isolate. Discs containing the following antibiotics were tested: Polymyxin B, Erythromycin, Chloramphenicol, Rifampicin, Ampicillin and Gentamycin [6].

3. RESULTS

Water pollution due to presence of metals has become one of the most serious environmental problems today. Biosorption, using inactive/dead biomaterials such as bacteria, fungi, algae and industrial/agricultural wastes, is regarded as cost-effective technology for the treatment of metal-bearing wastewaters. In recent years, several biosorbents have been investigated, but the bacterial biomass has since proven to be the most effective and promising biosorbent for wide variety of metals.

Sample collection and isolation

In this study, a total of 6 samples were collected from different untreated effluent and activated sludge samples collected nearby Chennai.

Screening of potential chromium resistant organism

A total of 5 different isolates were selected as potential chromium resistant bacteria based on their growth observation in chromium (100mg ml⁻¹ concentration) amended nutrient medium. The isolated Chromium resistant microorganism was subjected for further studies.

Analytical estimation of chromium

Chromium analysis and uptake capacity Cr (VI) was analyzed spectrophotometrically after complexation of metal ion with 1, 5-diphenylcarbazide. Absorbance was recorded at 540 nm, using Shimadzu (Beckman DU40) spectrophotometer using standard method.

All the five different isolates were grown and harvested and the dried biomass was used for the biosorption experiments. Among the 5 different isolates, one was selected as a prospective strain for the future studies based on their biosorption efficiency.

Optimization parameters for biosorption efficiency

The environmental parameters show great influence on the biosorption process. The important parameters like Temperature, pH, initial metal concentration and initial biomass concentration are considered as the essential parameters for the biosorption of Cr (VI).

Effect of pH on biosorption

The pH of the metal solution plays a crucial role in the chromium biosorption. Based on the results it was observed that optimal pH for biosorption of Cr (VI) ions is 2.0 (Fig 1).

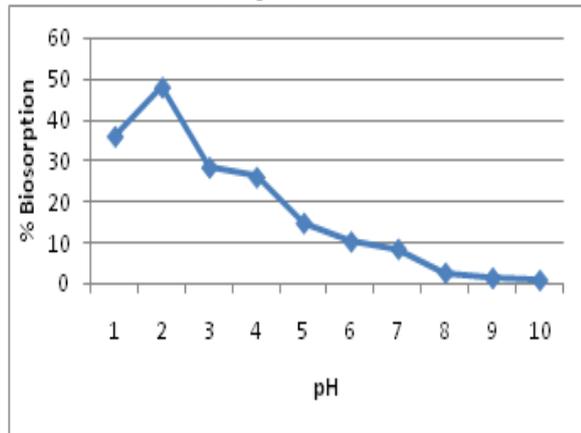


Fig 1: Effect of pH on biosorption of chromium

Effect of temperature on biosorption

Temperature plays a vital role in the biosorption of metal ions. Therefore, experiments were performed to examine the temperature dependency of Cr (VI) sorption by the dead biomass. Based on the results, maximum biosorption of chromium was observed at 27°C. (Fig 2).

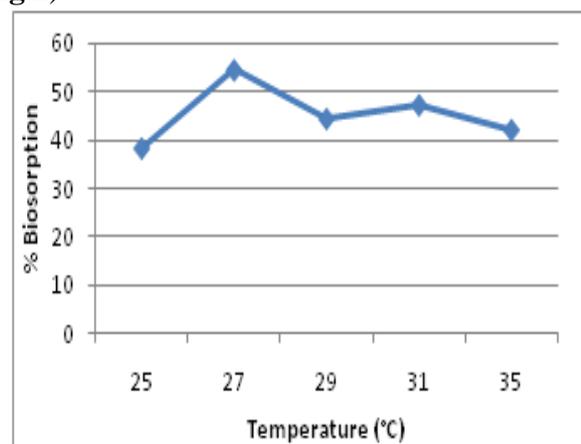


Fig 2: Effect of temperature on biosorption of chromium

Effect of incubation time on biosorption

The effect of incubation time on biosorption of Cr (VI) ions was analyzed. This study noticed that biosorption efficiency of increased with increase in biomass concentration. Maximum biosorption was observed when the test conducted at 24 hours incubation period (Fig 3).

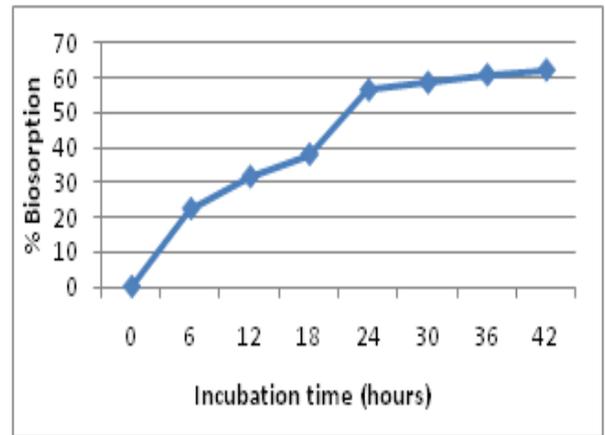


Fig 3: Effect of incubation time on biosorption of chromium

Effect of biomass concentration on biosorption

The effect of biomass concentration of Cr (VI) ions were examined by varying biomass dosage from 1 mg to 10 mg⁻¹. This study noticed that removal efficiency increased with increase in biomass concentration (Fig 4).

Effect of initial metal concentration on biosorption

Biosorption experiments were conducted by taking different initial metal concentrations by fixing all the parameters such as biomass concentration, pH, temperature and time (Fig 5).

Effect of carbon source on biosorption

Different carbon sources were studied for maximum biosorption of Cr (VI). As it is seen from result, Sucrose is more efficient in biosorption (Fig 6).

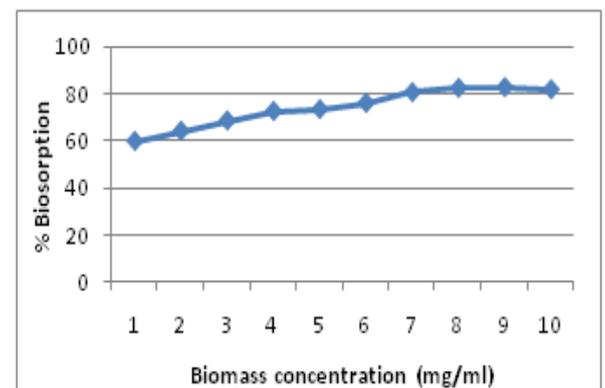


Fig 4: Effect of biomass concentration on biosorption of chromium

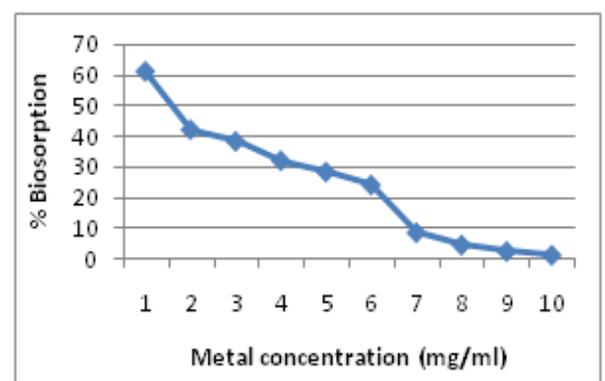


Fig 5: Effect of initial metal concentration on biosorption of chromium

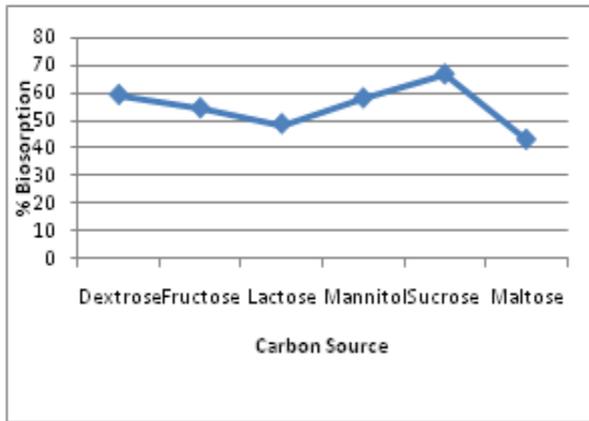


Fig 6: Effect of carbon source on biosorption of chromium

Characterization of biosorbent

Fourier transform-infrared spectroscopy

The FTIR spectra of control biosorbent (without chromium) and chromium treated biosorbent (with Chromium) were shown in (Fig 7 & 8). In control biosorbent adsorption bands were observed at 3389.75 cm⁻¹, 2108.58 cm⁻¹ and 1638.90 cm⁻¹.

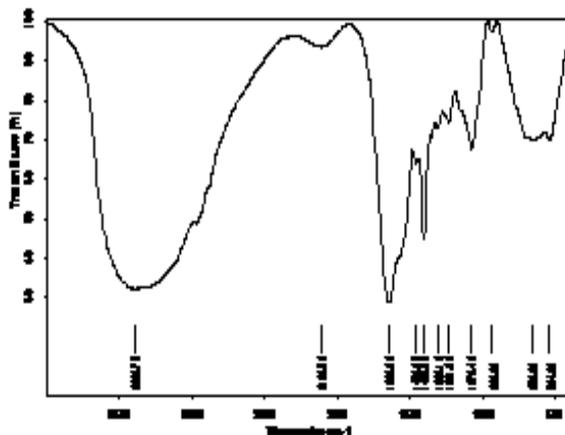


Fig7: Biomass without chromium

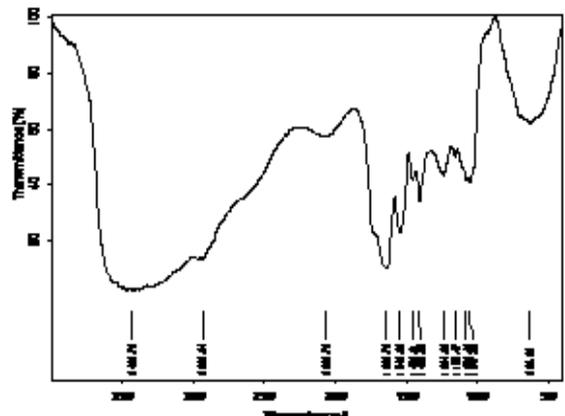


Fig 8: Biomass with chromium

In Chromium treated biosorbent there were adsorption bands at 3430.72 cm⁻¹, 2932.84 cm⁻¹, 1638.72, 1542.89 cm⁻¹, 1155.47 cm⁻¹ and 1047.63cm⁻¹. The absorbance peak values obtained were compared with earlier reports. The values were analyzed using reported literatures.

Scanning electron analysis

The SEM image of Unloaded and metal-loaded biomass biosorbent were shown in (Fig 9 & 10). Over the biosorption period, the morphology of the bacteria had undergone remarkable physical disintegration which can be observed in the micrograph.

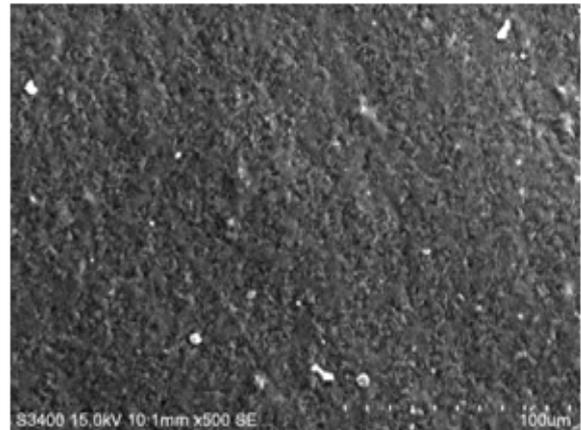


Fig 9: Unloaded biomass biosorbent

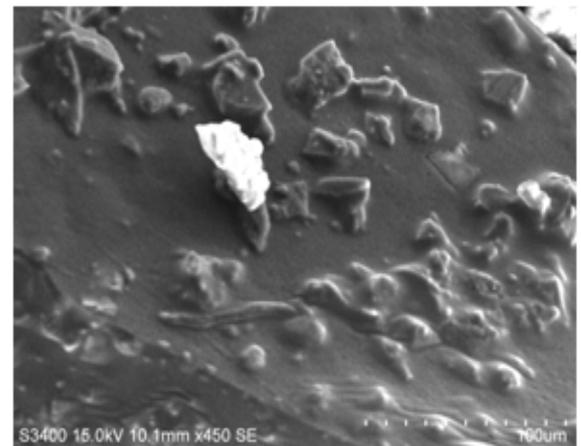


Fig 10: Metal-loaded biomass biosorbent

Immobilization

Biosorption studies were performed by dispersing 0.1 % beads containing biomass in 25 ml of the solution containing 1mg/ml of metal concentration. The result shows that selected isolate has 73% biosorption capacity.

Desorption studies

Biosorption is a process of treating pollutant-bearing solutions to render it contaminant-free. However, it is also necessary to be able to regenerate the biosorbent. Desorption is of utmost importance when biomass preparation/generation is costly, as it is possible to decrease process cost and the dependency of the process on a continuous supply of biosorbent. A successful desorption process requires the proper selection of elutants which strongly depends on the type of biosorbent and the mechanism of biosorption.

Desorption studies were carried out with biomass loaded with Cr(VI) was treated with 0.1 M NaOH

at room temperature (27°C) with constant shaking for 2 hours. The release of sorbed Cr (VI) ions was up to 81.3% using NaOH was achieved.

Antibiotic disc assay

The isolated organism was tested for antibiotic sensitivity and appeared to be susceptible to Chloramphenicol followed with Gentamycin. The selected isolate shows resistant to the rest of the antibiotics.

Molecular Studies

Isolation of genomic DNA

The 24 hour old bacterial culture of *Bacillus licheniformis* strain BIOS PTK grown in Luria Bertani medium at 37 °C under aerobic conditions at 100 rpm were collected and isolated the respective genomic DNAs was isolated. The isolated genomic DNA of the *Bacillus licheniformis* strain BIOS PTK was visualized under UV light after agarose gel separation.

PCR amplification of 16S rRNA gene

Polymerase chain reaction was performed in Thermocycler (PTC – 100 TM Programmable Thermal Controller, USA) to produce multi-copies of the specified DNA. The PCR reaction was allowed for 30 cycles for amplification of 16S rRNA gene. Then the PCR product was run on 2 % agarose gel electrophoresis along with 2 Kb DNA ladder mix and visualized under UV light.

Sequence analysis of PCR-amplified product

The nucleotide sequence of PCR products of both forward and reverse sequences of the *Bacillus licheniformis* strain BIOS PTK16S rRNA gene ~ 1446.

Molecular identification of *Bacillus licheniformis* strain BIOS PTK

The potential isolate, *Bacillus licheniformis* strain BIOS PTK was ascertained its systematic position based on 16S rRNA sequence analysis and with the aid of computational programme, BLAST homology analysis was also carried out to compare with other 16S rRNA sequences available in the GenBank of NCBI. It revealed that the sequence of *Bacillus licheniformis* strain BIOS PTK.

CONCLUSION

Our study also shows that *Bacillus licheniformis*, an effluent isolate, shows maximum biosorption ability towards the reduction of Chromium (VI) ions. Many scientific studies are currently under way and contributions to welfare are welcome in this world which grows each second and which needs to be in equilibrium with so much progress. Some pollution seems inevitable, and one can wonder what one should do to minimize it?

Human populations need methods and technologies to clean waters and diminish the environmental dangers related to progress. Biosorption can be a solution to clean the environment contaminated by heavy metals. When matter was first tamed, nobody could foresee how many problems humans would have to face in the future.

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