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Review Of Research

# BIOREDUCTION OF CHROMIUM (VI) BY HALOALKALIPHILIC BACILLUS SPECIES STRAIN ISOLATED FROM ALKALINE LONAR LAKE

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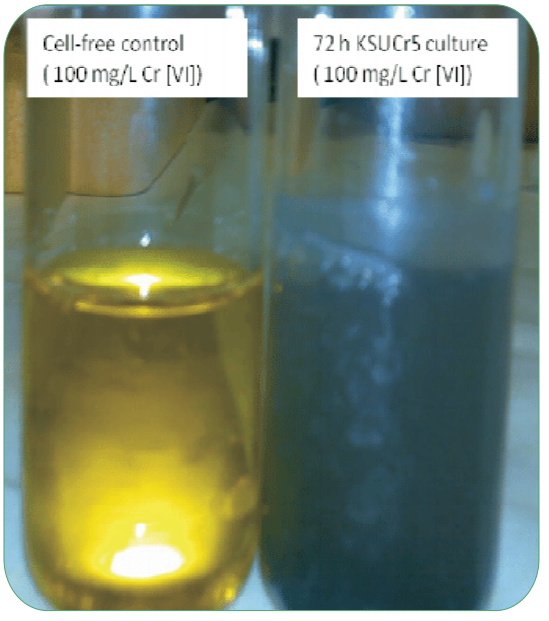


## A\BSTRACT

*Alkaline Lonar Lake in India having a novel environment, shaped by shooting star affect on basaltic shake, arranged in Buldhana District of Maharashtra State, India. The hexavalent type of chromium is the most dangerous and cancer-causing and delivers wellbeing risky impact. Consequently endeavor was made to disengage chromium remediating microscopic organisms from halophilic environment, for example, Lonar Lake. In these studies, water, silt and matt specimens were gathered from soluble Lonar Lake and vaccinated in Nutrient juices containing K2Cr2O7 (100µg/mL). Disengage was portrayed by social, morphological and biochemically and 16S rRNA quality sequencing distinguished the living being as Bacillus flexus (ADA3). The chromium decrease capacity of the Bacillus flexus (ADA3) was evaluated by the Spectrophotometric strategy for Di-phenyl carbazide. Bacillus flexus (ADA3) diminished 81% of chromium after 96 h of brooding individually. Aftereffects of this study demonstrated that, the Bacillus flexus (ADA3) was observed to be exceedingly productive chromium reducer and could use for bioremediation on contaminated destinations.*

**KEYWORDS :***Lonar Lake, Hexavalent Chromium, Bacillus flexus and Di-phenyl carbazide.*

## INTRODUCTION

Substantial metals found in wastewater are unsafe to nature and their consequences for organic framework are extremely serious. A proficient and monetary treatment for their evacuation and reuse should be produced. Microbial metal bioremediation is a proficient methodology because of its minimal effort, high effectiveness and eco-accommodating nature. As of late advances have been made in comprehension metal microorganism connection and their application for metal detoxification (Smrithi and Usha, 2012). The extensive application of chromium in industries particularly leather, tanning industries leads to the formation of chromium-contaminated soil and ground water which pose a serious threat to the living biota particularly to human health. Chromium is a potent pollutant which is mutagenic, carcinogenic and teratogenic in human being (Petrilli and Flora, 1977, Gale, 1978). Among the various heavy metals, hexavalent chromium (VI) has been found in harmful in surface water due to contami¬nation introduced from industrial pollution.

Chromium ex¬ists in the environment in several diverse forms

such as trivalent Cr (III) and hexavalent Cr (VI) of which hexavalent chromium Cr (VI) is carcinogen (Cervantes *et al*, 2001) and also cause dermatitis, damage to liver, kidney circulation, nerve tissue damage and death in large doses (Das and Mishra, 2008, Malik, 2004). Because of the wide range of exposure of chromium in the environment the need of finding potential microbes having potential Chromium immobilization property. Few microorganisms in the environment have been identified as potential chromium removal capacity (Tambekar *et al*., 2014, Kamika I and Momba MB, 2013, Sathya R and Sankar P, 2010, Srinivasan *et al.*, 2014 Chandhuru *et al*., 2012; Mehta NJ and Vaidya VK, 2010). Microbes will adapt quite rapidly and grow at extreme condition using hazardous compounds as energy sources in extreme conditions in waste streams. Such extreme condition found in Lonar Lake, which situated in Buldhana district of Maharashtra state, India, ranks third in the world based on diameter and its high alkalinity (pH 10.5) (Tambekar et al., 2010).

## MATERIALS AND METHODS

**Collection of Samples:** Total twelve sediment, matt and water tests were gathered from four distinctive area of basic Lonar Lake amid rainstorm season by utilizing disinfected spatula. All specimens were marked and kept in clean plastic jug (water test) and zip bolt sack (residue and matt example) at 4˚c until examination.

**Enrichment of samples:** Improvement of tests: All twelve residue, matt and water tests were blended quickly in partitioned sterile holders for confinement of Cr lessening microscopic organisms immunized in 250mL Erlenmeyer's jar containing disinfected Nutrient stock medium (pH10) containing of K2Cr2O7 (100µg/mL). After 72 h of brooding 10 mL culture soup was rehashed subcultured in crisply arranged supplement medium having same organization for five times for improvement of bacterial culture.

**Isolation, biochemical and identification characterization:**Detachment, biochemical and distinguishing proof portrayal: After advancement, the seclusion was made by vaccinating the way of life soup on strong supplement agar plate with pH 10 by streak plate technique**.** The well isolated and morphologically distinct colonies from the plate of sediment, matt and water samples were selected and stock cultures were prepared for further analysis. Isolated bacterial strain was identified by cultural, morphological and biochemical was identified by commercially available Hi-media rapid detection kit KB009 and KB012. The bacterial strain was also identified by 16S rRNA gene sequence analysis from Agharkar research institute, Pune.

**Di-phenyl carbazide assay for Cr (VI) reduction:** For the determination of chromium in resent study, diphenyl carbazide (DPC) method of Cr estimation was adopted with the help of Spectrophotometer and AAS. Chromium (VI) reacts with DPC to form reddish violet complex. The reaction is selective for chromium and very sensitive. One tenth to six microgram of chromium could be determined with an error less than 2 % (Tsunenobu et al., 1978). Standard graph for estimation of chromium was first prepared by using different concentration of chromium 20µg/mL to 120µg/mL by using DPC. The estimation method of Cr (VI) (APHA, 2012) by specific colorimetric reagent, 1, 5-diphenyl carbazide (DPC) (500 mg in 100 mL Acetone; 10% $H_2SO_4$, to give pH 2 ± 0.5). Volume was made up to 100 mL in borosil volumetric flasks. Cr estimated by taking the absorbance at 540nm on UV-VIS spectrometer (make Systronics) and concentration was also tallied on AAS instrument (APHA, 2012)

**Table: 1. Cultural, morphological and biochemical characteristics of chromium reducing bacteria isolated from alkaline Lonar Lake**

| TEST | RESULT | TEST | RESULT | TEST | RESULT |
|---|---|---|---|---|---|
| Shape | Rod | Catalase | + | Cellibiose | - |
| Colour of colony | white | Oxidase | + | ONPG | + |
| Gram staining | +ve | MR | - | Esculin | + |
| Texture | Sm | VP | - | Nitrate reduction | + |
| Arrangement | S | Citrate | - | Lactose | - |
| Motility | +ve | Xylose | + | Arginine | + |
| **Growth at different temperature** | | Ornithine | - | Sucrose | + |
| 30ºC | + | Xylitol | - | Maltose | + |
| 40ºC | + | Sorbose | - | Fructose | + |
| 50ºC | + | Lysine | - | Dextrose | + |
| **Growth at different pH** | | D-Arabinose | - | Mannose | + |
| pH 7 | + | Glucose | + | Melibiose | + |
| pH 8 | + | Galactose | + | Glycerol | - |
| pH 9 | + | Raffinose | + | Salicin | - |
| pH 10 | + | Trehalose | + | Dulcitol | - |
| pH 11 | + | Mannitol | + | Inocitol | - |
| **Growth at different salt conc.** | | Adonitol | - | Sorbitol | - |
| 9% | + | Saccharose | - | L-Arabinose | - |
| 10% | + | Esculin | - | Rhamnose | - |
| 11% | + | Erythritol | - | Sodium Gluconate | - |

**Note**:- + = Positive; - = Negative; R= Rod; S= Single; Sm=Smooth

## RESULTS AND DISCUSSION

In the present investigation, attempt was made to isolate chromium reducing microorganisms from Alkalophilic environment such as Lonar Lake. There are certain microorganisms which have been reported by various researchers but detail studies on the bioremediation of chromium from Lonar Lake were yet not to be done. Hence this study focuses on the study of isolation of chromium reducing bacteria for bioremediation of chromium. A total of twelve water, matt and sediment samples were collected from the alkaline Lonar Lake in the season mansoon and well isolated and morphologically distinct colonies from the plate was selected and Gram positive short rod and motile bacteria was isolated and identified. The biochemical characteristics of the isolate Bacillus flexus (ADA3) was done by the commercially available Hi-media rapid detection kit KB009 and KB012 (Table 1).

The isolate ADA-3 was also identified by 16S rRNA gene sequencing from Agharkar research institute, Pune. The result of 16S rRNA gene sequencing showed that the organism was *Bacillus flexus* (ADA3) (Table 2). In the present study, the isolate when studied for chromium estimation then the rate of reduction and percent reduction of chromium was found to be 0.843 and 81% µg/mL after 96 h. In case of pH the chromium reduced on 10 pH was maximum (Fig.1and 2). The effect of various environmental parameters on chromium reduction efficiency was also studied and it was found that the optimum temperature for organism is 400C for reduction of chromium, while on 30$^0$C and 50$^0$C, the percent reduction and rate of reduction slows down (fig.3and 4). In case of pH the chromium reduced on 10 pH was maximum (Fig.1and 2).and it was found that the optimum salt concentration of chromium reduction on 10% was maximum (fig.5 and 6). Farah et al.,(2010) revealed that the isolates *B. pumilus, Staphylococcus* species and *Alcaligenes faecalis* reduces Cr6+ 95%, 91% and 97% within 24 h from the medium containing 100µg/ml chromium. Wani et al,(2007) isolated the chromium (VI) degrading *bacterium Burkholderia cepacia* from alkaline environment of Lonar Lake and the isolate was

resistant to 1,000 ppm concentration of chromium.

**Table 2 : The 16S rRNA gene sequencing closest phylogenetic affiliation, pair similarity and ribosomal database project report of isolated chromium bioremediating organism ADA 3 from Lonar lake**

| Strain Designation | Strain Designation | Strain Designation |
|---|---|---|
| ADA 3 | ADA 3 | ADA 3 |

Sequence

TTCCGCAAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCG
TAAAACTCTGTTGTTAGGGAAGAACAAGTACAAGAGTAACTGCTTGTACCTTGACGGTACCTAA
CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATC
CGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAGCGGAATTCC
ACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGT
CTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCA
TTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCC
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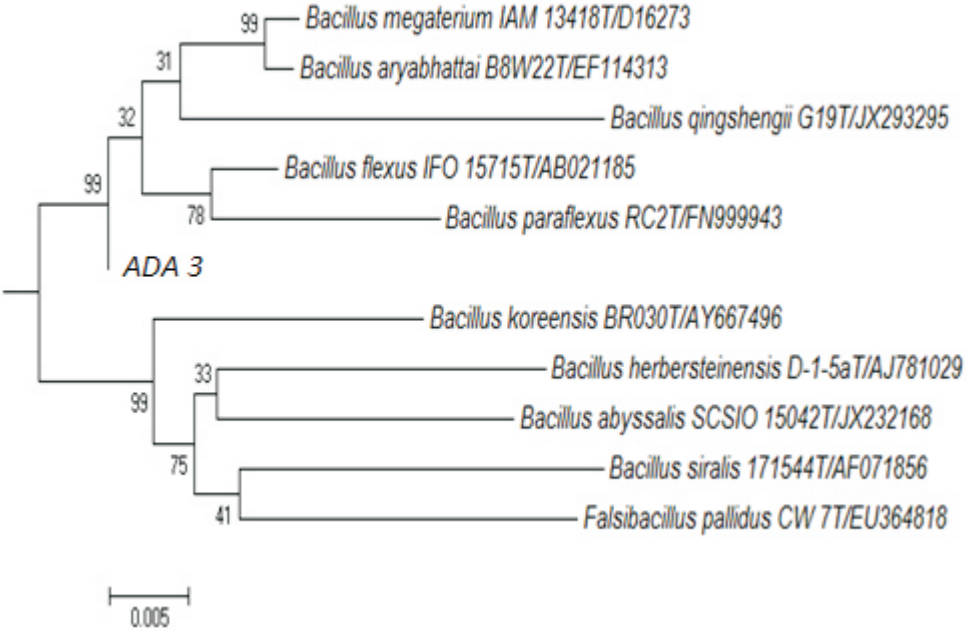
CATCCTCTGACANCTCTAGAGATAGAGCGTTCCCCTTC




## CONCLUSION

Reduction, detoxification and possible remediation chro¬mium by using various microorganisms has been the top¬ic of scientific interest for a number of decades. A large number of natural and synthetic organic compounds are biodegradable by microorganisms as part of their normal metabolism for energy and growth. From the data, the strain *Bacillus flexus* (ADA3) was isolated from Lonar Lake showed the potential to reduce, detoxify and possibly re¬mediate chromium effectively and eco-friendly by which it reduces the pollution from water. From the study, it can be concluded that *Bacillus flexus* (ADA3) can be ex¬ploited for bioremediation of toxic hexavalent chromium to trivalent chromium from the industrial effluent and other.

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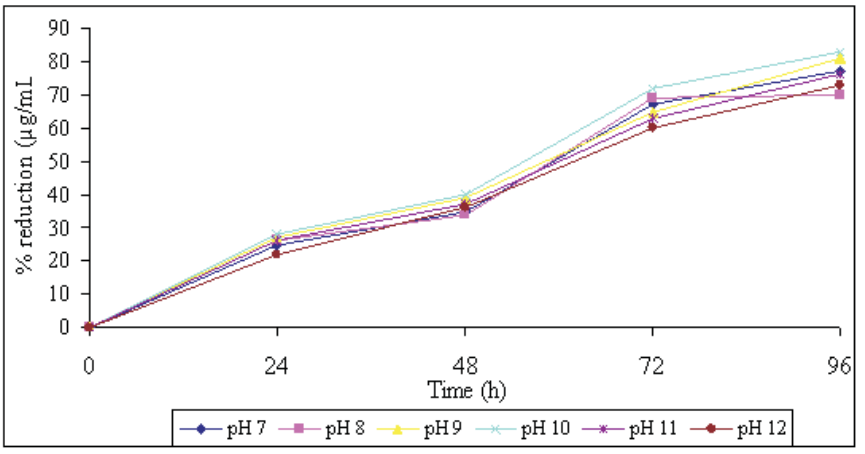


**Fig.1**: Effect of pH on % reduction of chromium by *Bacillus flexus* (ADA3)

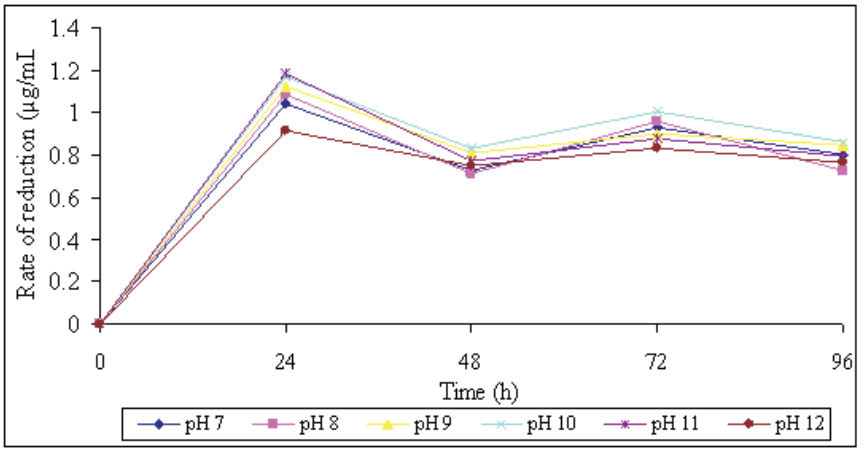


**Fig.2**: Effect of pH on rate of reduction of chromium by *Bacillus flexus* (ADA 3)

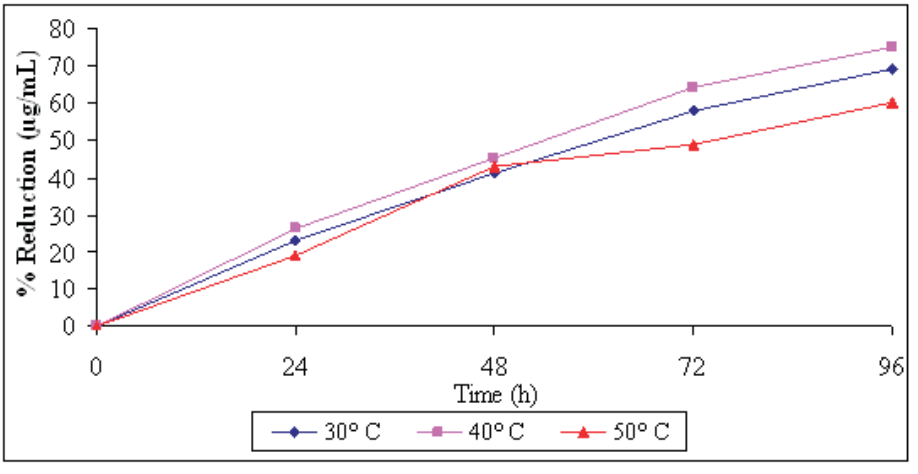


**Fig.3**: Effect of temperature on % reduction of chromium by *B. flexus* (ADA3)

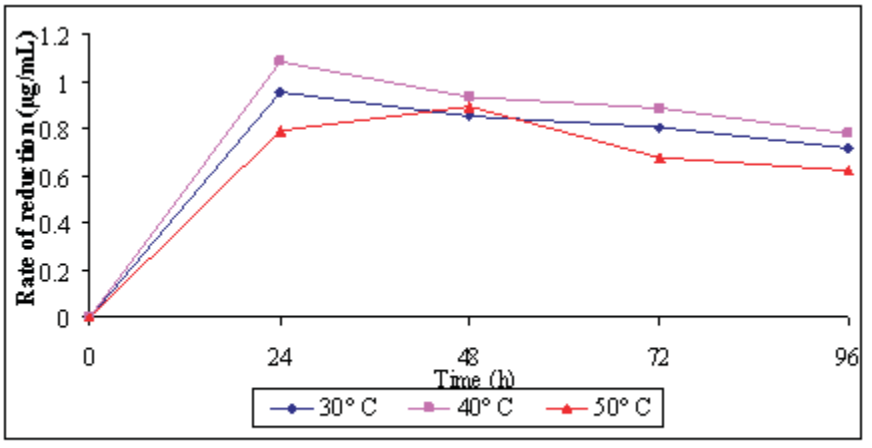


Fig.4 : Effect of temperature on rate of reduction of Cr by *B. flexus* (ADA3)

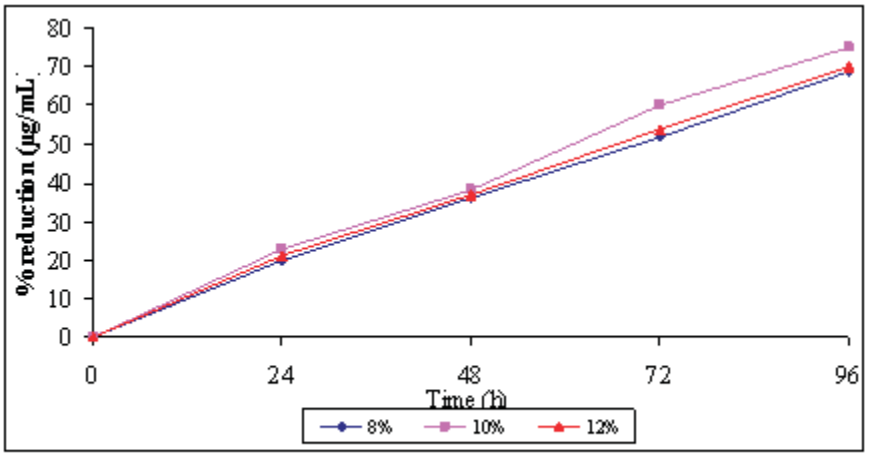


Fig. 5: Effect of salt concentration on % reduction of Cr by *B. flexus* (ADA 3)

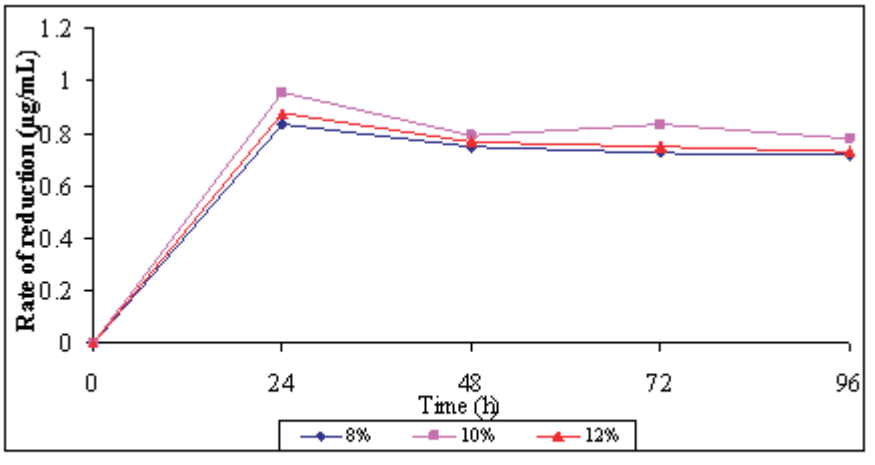


Fig. 6: Effect of salt concentration on % rate of reduction of chromium by *Bacillus flexus* (ADA 3)

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