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Isolation and Characterization of Hexavalent Chromium Reducing Bacteria for Application in Microbial Fuel Cells

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ABSTRACT

Wastewater from several businesses that produce paints, steel, tannery products, dyes, and chrome-plated items contains hexavalent chromium (Cr(VI)). The main contributing factors of pollution concentrations in water bodies is the wastewater that tannery businesses dump, which includes organic pollutants and heavy metals, particularly Cr (VI). The harmful consequences of Cr(VI) on humans include eczema, allergies, ulceration, respiratory tract problems, lung cancer, as well as genotoxic and mutagenic effects. Using bacteria to biotransform hexavalent chromium to trivalent chromium (Cr(III)) is a practical strategy with proven viability in bioremediation. Following their isolation from the tannery industry's raw effluent, bacterial strains were investigated biochemically and molecularly. Based on this study, it was concluded that the microorganisms resistant to Cr (VI) were *Bacillus albus* and *Bacillus australimaris*. In a two chambered microbial fuel cell reactor, the isolated Cr (VI) resistant bacteria will be employed as electrogenic bacteria with SPEEK *(Sulphonated polyether ether ketone) as* PEM (proton exchange membrane), that can synergistically aid in the reduction of hexavalent chromium and green energy generation.

Key Words	Heavy Metal, Hexavalent Chromium, Tannery, Bacteria, Environmental Pollution
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INTRODUCTION

Cr(VI) is widely used in a number of industrial processes, such as tanning, wood preservation, and electroplating. Cr(VI) is available commercially as potassium chromate (K_2CrO_4) and potassium dichromate ($K_2Cr_2O_7$). Hexavalent chromium is found in a lot of the solid and liquid waste that is produced by the chromium manufacturing sector. Before releasing these wastes into the environment, they must be treated (Alam *et al.*, 2020). Compounds containing Cr(VI) are very soluble in water, poisonous, and carcinogenic to mammals. Trivalent chromium, on the other hand, is thought to be harmless as it forms insoluble oxides, hydroxides in soil and water systems at pH values greater than 5.5. One of the exciting new technologies that is anticipated to be crucial to the clean-up of waste sites is bioremediation. The goal of the biotreatment or bioremediation technique is to immobilize Cr(VI) in the soil matrix by detoxifying it in the wastewater and reducing it to Cr(III).

Enzymatic redox reactions are a common metabolic activity in microorganisms. Reactions that take place on the surfaces of bacteria can also decrease Cr(VI) non-metabolically (Kapahi and Sachdeva, 2019). Kapahi and Sachdeva (2019) have proposed this as the main reduction mechanism in natural geologic environments. Intracellular precipitation is a third method of Cr reduction (Rahman and Thomas, 2020; Tang *et al.*, 2021). The first process, in which Chromium is metabolically reduced in the presence of abundant electron donors, has been the subject of the majority of research (Lin *et al.*, 2020). Chemical reducing agents that necessitate biological interactions encompass the utilization of lactic acid, molasses, exclusive formulations like cheese whey and the Hydrogen Release Compound (HRC). These substances give off carbon dioxide into the atmosphere, but in an anaerobic environment, they need to undergo biological changes in order to produce hydrogen. Both aerobic and anaerobic routes can be used by bacteria to enzymatically decrease Cr(VI) (Kalsoom, Batool and Jamil, 2021). The biological mechanisms are in competition with other nonbiological routes for Cr reduction (Niu *et al.*, 2024; Gu *et al.,* 2024). Since biological reduction is sluggish in anaerobic environments, abiotic reduction by iron or hydrogen sulfide is anticipated to predominate. Only in aerobic settings can microbial decrease take on kinetic significance. The prime factors affecting the reduction rate are the quantities of oxygen in the system, pH, and geochemical conditions. The environment is always full of microorganisms. Research is still needed to identify their role in Cr reduction.

Pollution in the environment poses a major threat to the world. The magnitude of this problem seems to increase every day. Major cause of this issue is urbanizations and industrialization. This alters flow of substance in nature and instils chemicals that constitute the water bodies and its surrounding environment like soil, flora and fauna. Water pollution tops among all pollution in the present modern world (Solanki *et al.*, 2022). Wastewaters from industries heavily contaminates plants, human and animal lives with toxic heavy metals. Bioaccumulation in aquatic bodies and subsequently reaching the human tissues through ecosystem has been reported by many scientists in the recent years (Garg, Tripathi and Srinath, 2012). Chromium

pollution is mainly caused by tanning industries. More than 80% of the 2500 tanneries in India deal with chromium which gets released in the surrounding environmental bodies eventually (Shukla, Rai and Dubey, 2009). Chrome from high exhaust of tanneries can contaminate water bodies with up to 500-1000 mg Cr(VI) (Aravindhan *et al.*, 2004). Hence disposal issues of the same is of great concern to Environmental Protection Agency (EPA) and World Health Organization (WHO). Presence of chromium in the habitat affects optimal functioning of the ecosystem and impose economic burden. 0.1 mg/1is the permissible limit for chromium allowed by the pollution control board. In industries dealing with hide processing release around 250 Kg of solid wastes and 100 Kg of wastewater. Among the various tanning processes shaving of chrome leather and splitting of chrome takes 40% of the production process. This generates a lot of wastes in the form of chromium. Trivalent form of chromium is found to be less toxic when compared to its hexavalent counterpart. Tanning industry uses approximately 6000 tonnes of chromium of which 40% is discharges in to surrounding as waste. When chromium is used to tan hides the end product seems to have better mechanical resistance, good suitability for dyeing and exceptional hydrothermal resistance (Shaibur, 2023). Workers exposed to chromium along with respiratory illness are also prone to dermatological complications like 'chrome holes'. Severe cases lead to morbidity by 40% of which 16% is due to respiratory illness (Kasim *et al.*, 2014). Dry cough attributes to 5.6%, irritation in the throat by 3.6% and congestion in the lungs by 3% (Tremper, 2005).

The microbial consortia were isolated from the raw effluent of the tannery industry situated in the North Tamil Nadu district and was used to reduce the hexavalent chromium levels in the chrome tanning effluent. The effectiveness of the consortia was analysed in this work and the same will be used as inoculant for microbial fuel cell experiments wherein SPEEK (sulphonated polyether ether ketone) will be used as PEM (proton exchange membrane) (Fig 1)

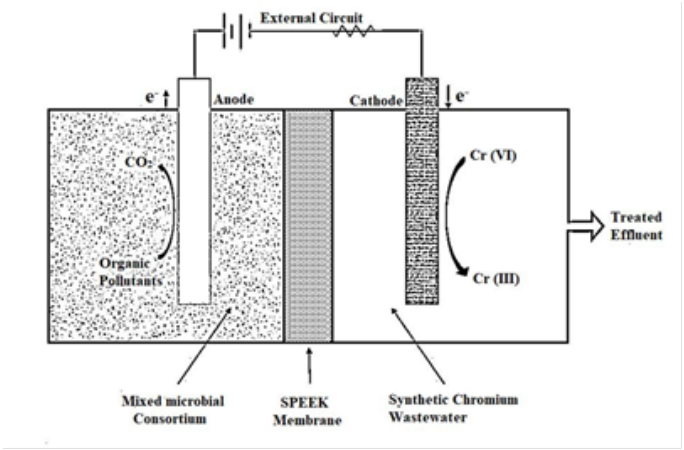

Fig.1: Microbial Fuel Cell

The present study utilizes a group of microorganisms isolated from the raw effluent of the tannery industry situated in the North Tamil Nadu district and is used to reduce hexavalent chromium into trivalent. The previously reported studies exploited a variety of other methods such as using a reductant like Epigallocatechin gallate (EGCG), and other chemicals like

(Polystyrylmethyl) trimethylammonium borohydride, sodium metabisulphite, catalytic membrane reactors containing palladium, and using bacteria like *Halomonas maridiana* EA1

MATERIALS AND METHODS

Sample collection

The sterile nutrient broth (NB) was used to serially dilute the effluent sample (1 ml) from 10−1 to 10−5 dilution before plating it on nutrient agar (NA) (HIMedia). The plates were incubated at 37°C for 24 hrs. The well-isolated colonies were picked up using sterile inoculation loop and further examined for the growth on effluent-containing plates. Tannery effluent-based agar medium was prepared, in which tannery effluent was the sole carbon source (Sanjay *et al.*, 2020). The tannery effluent was (1L) filtered and 15g of agar powder (HiMedia, Mumbai) was added for solidification. Then the media was sterilized, cooled and the agar plates were prepared which were later inoculated with the isolated bacteria. After 24 hrs. the bacterial colonies were characterized for its shape, size, colour, margin and gram staining. Glycerol stocks of the culture was maintained in NA slants and stored at 4 °C.

Biochemical characterization of bacteria

The physical and biochemical features of the isolated bacterium were carried as per the Bergey's Manual of Determinative Bacteriology was used to characterise the chosen bacterial isolate. Morphological traits were seen for each bacterial colony after a 24-hour incubation period at 37°C. After Gram staining, the colony morphology was investigated under a microscope and biochemical analysis like Kovacs- indole test, methyl red test, Voges-Proskauer, endospore, catalase and starch hydrolysis tests were done on the bacterial isolate.

DNA isolation

Samples that were modified using the normal salting-out process were used to isolate DNA. 10% of the 10L SDS (Sigma Aldrich) and 500L of Solution 1 were added to the sample tube. Make use of a sterile homogenizer to blend the bacterial sample. Additionally, Proteinase K of 5 litres (20 mg/mL) was added. For easier digestion, the mixture was maintained at 55°C for two hours in a water bath with sporadic mixing and rapid vortexing. The sample was placed on ice for ten minutes following full digestion. 250 µL of Solution 2 was added to this, and it was mixed by repeatedly inverting it. The samples were then refrigerated for five minutes on ice. The samples were subjected to centrifugation (Eppendorf 5804R) for 15 minutes at 8000 rpm. Following that, roughly 500 litters of clear supernatant were gathered and placed into fresh, labelled 1.5 mL tubes. To precipitate the DNA, twice as much hundred percent molecular biology grade ethanol (Sigma Aldrich) was added. After that, the samples were centrifuged for 15 minutes at 11,000 rpm. Following that, 500L of 70% ethanol was added for washing, and the supernatant was disposed away. Sample was spun for five minutes at 11000 rpm. Remove the supernatant with care, then let it air dry at room temperature. DNA that had partially dried was reconstituted in 100L of elution and kept at -20°C.

Molecular region

In order to confirm the bacterial species using molecular means, the standard 16s rRNA gene was chosen. Using universal primers, PCR were used for 16S rRNA gene amplification.

Primers

Universal 16S rRNA gene primers Forward 27F 5′- AGAGTTTGATCMTGGCTCAG - 3′ Reverse 1492R 5′- GGTTACCTTGTTACGACTT - 3′ were used for the amplification.

Polymerase chain reaction condition

The polymerase chain reactions were conducted using thermal cycler (Thermo Fisher, Moldel. No. GeneAmp 9700). A molecular weight marker (a 100 bp ladder) was used to determine the molecular weight of the products amplified using 1.5% AGE (agarose gel electrophoresis).

Molecular identification by 16S rRNA gene sequence

The samples that were altered from the conventional salting-out process were used to extract the genomic DNA of the bacterial isolates. For the bacterial species' molecular confirmation investigations, the standard 16s rRNA gene was chosen. Using universal primers, PCR were used for 16S rRNA gene. Amplification. Primers for the whole 16S rRNA gene were chosen for amplification, encompassing all variable regions, based on prior research. Forward 27F and 5′-AGAGTTTGATCMTGGCTCAG - 3′, Reverse 1492R and 5′-GGTTACCTTGTTACGACTT - 3′ are the primer name and sequences, respectively. A molecular weight marker (a 100 bp ladder) was used to determine the molecular weight of the amplified products using 1.5% AGE.

Sequencing the amplified product

Sequencing using BigDye Terminator v3.1

The sequencing process was conducted in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA).

Sequence Analysis.

The sequence quality was verified using Applied Biosystems' Sequence Scanner Software v1. MEGA 7 was used for sequence alignment and any necessary modification of the acquired sequences.

BLAST analysis

It was determined that the amplified sequences match the 16S rRNA by utilizing the similarity index created by the NCBI's BLAST program. The study's species were categorized according to their greater percentage of similarity to the reference species.

Effect of Chromium (VI) on bacterial growth

In 10 ml of nutrient-rich broth, the effects of several dosages of Cr(VI) on the resistance of the bacterial growth were examined in triplicate at 37 °C and 200 rpm in a water bath. The optical

density (OD) (Shimadzu, UV-1900i model double-beam) of the culture at 600 nm was used to track the growth.

Chromium(VI) Assay

Bacterial growth and Cr(VI) reduction time courses were assessed in a shake flask culture using 100 ml of nutrient broth (HiMedia) at 37°C and 200 rpm, using 20 mg/l $K_2Cr_2O_7$. 2.0 ml of samples were taken out every 0.5 or 2 hours. The OD at 600 nm was used to track the increase. The samples were centrifuged for five minutes at 10,000 rpm, and the supernatants were tested for the presence of leftover Cr(VI).

Determination of Bacterial Resistance or Tolerance to Cr

Using a broth dilution approach, a number of bacterial isolates that showed a significant capacity to detoxify or decrease Cr were investigated to ascertain their tolerance or resistance to the element. By using this method, bacterial cells with a density of 106 cells/mL were cultured in brain heart infusion broth supplemented with varying amounts of $K_2Cr_2O_7$ for 24 hours at 37°C and agitation at 100 rpm. With regard to the bacterial growth, the turbidity of the broth was evaluated at 600 nm using a UV-visible spectrophotometer. By examining the isolates that demonstrated a significant concentration of Cr shows good to moderate growth was observed.

RESULTS AND DISCUSSION

From NB plates containing Cr(VI), five distinct colonies were chosen and introduced with effluent obtained from a leather tannery common effluent treatment plant in North Tamil Nadu district. To test their tolerance to Cr(VI) ranging from 500 to 2,000 mg/l, the samples have been further inoculated in NB broth treated with various doses of Cr(VI). The highest possible level of tolerance was displayed by isolates T1 and T2, which grew well in the presence of 2,000 mg/l Cr (VI) (Fig. 2) while some isolates did not accelerate their growth rate over 1,000 mg/l Cr (VI) and hence the isolates T1 and T2 were chosen for further study. Hussain and Al-Saadi (2022) isolated certain bacteria that can with-stand or reduce Cr(VI) at values of 1,500–2,500 mg/l are considered as chromium resistant. Both biochemical and 16S rRNA gene sequence analysis were carried out to identify the isolates T1 and T2 (Table 1). The isolate T1 exhibited 59% identity with *Bacillusalbus* (Fig. 1) and isolate T2 showed 93% identity with *Bacillus australimaris* (Fig. 3) (Escamilla-Rodríguez, Carlos-Hernández and Díaz-Jiménez, 2021; Kookhaee, Bafroee and Jabalameli, 2022). Sanjay *et al.,* (2020) reported Bacillus sp. Play a major role in reducing hexavalent chromium isolated from tannery effluent samples and chromite mine soils respectively.

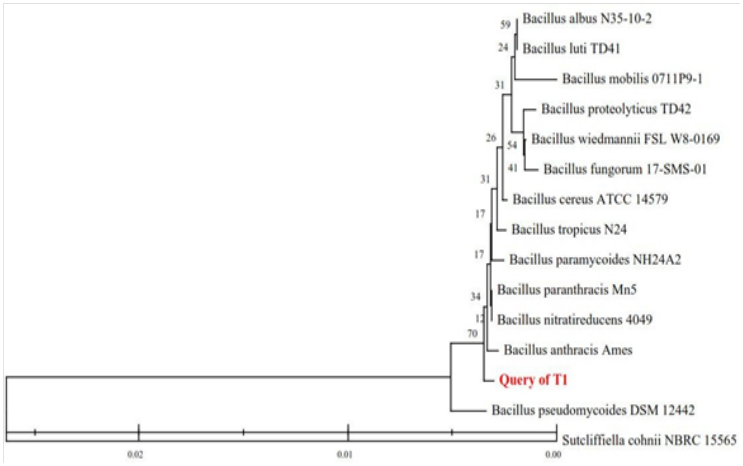

Fig 2: Evolutionary relationships of taxa (T1)

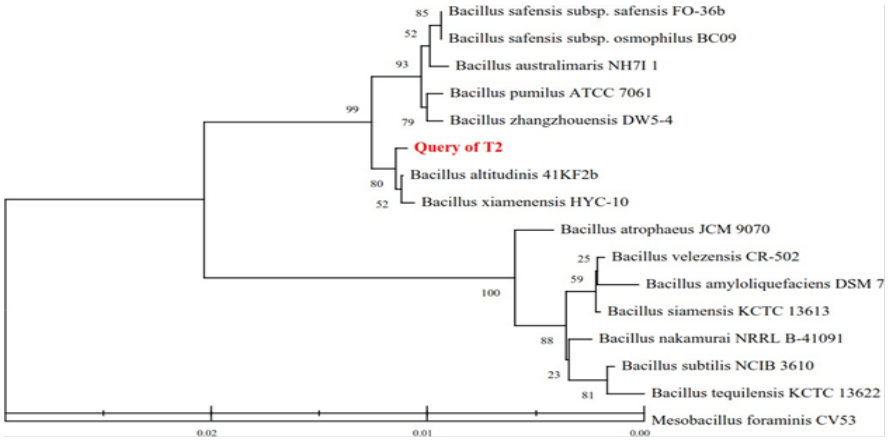

Fig 3: Evolutionary relationships of taxa (T2)

Table 1: Identification of chromium (VI)-reducing bacteria by 16S rRNA gene sequence analysis.

Strain name	Name of Bacteria	Percentage Identity
T1	*Bacillus albus*	59
T2	*Bacillus australimaris*	93

Isolation and characterization of bacteria

Identification of the isolated bacteria

The bacterial isolates that were chromium tolerant were identified using Bergey's Manual of Determinative Bacteriology (Sharma *et al.*, 2023). The selected isolates shared a common morphological and biochemical characteristics like size, shape, arrangements, Gram-positive, negative indole, negative MR (methyl red), positive catalase, positive citrate and positive starch hydrolysis (Table 2). The isolates were chosen for further identification after being identified as *Bacillus sp.* N35-10-2 and NH711 based on their morphological features.

Given samples were serially diluted from 101 to 109 and spread plated on LB agar medium. After 16 hours of incubation at 37°C, plates were subjected for observation. Two different morphologies were dominantly present in all dilution plates. Those 2 colonies were selected and subjected for molecular identification (Fig 4 and 5).

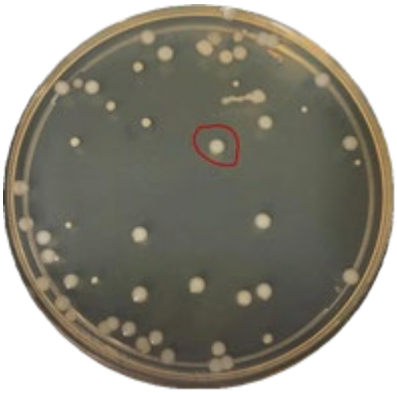

Fig 4: Plate 1 – T1 (Marked in red color)

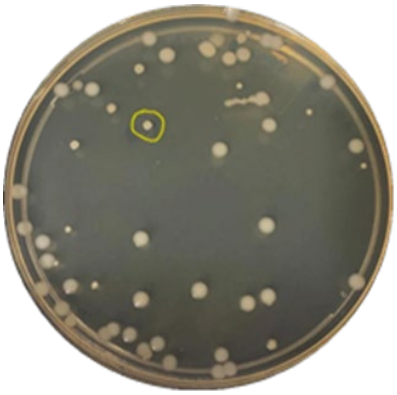

Fig 5: Plate 2 – T2 (Marked in yellow colour)

Table 2: Morphological and biochemical characteristics of isolated Cr reducing bacteria

Characteristics	**Observations**	
	T1	**T2**
Morphology and staining		
Gram's staining	Positive	Positive
Acid fast staining	Negative	Negative
Shape	Short rod	Short rod
Arrangement	Single	Single
Biochemical tests		
Indole	Negative	Negative
Motility	Positive	Positive
MR-VP	Negative	Negative
Catalase	Positive	Positive
Citrate	Positive	Positive
Starch hydrolysis	Positive	Positive

Oxidase	Negative	Negative

Determination of DNA quality

The amount of DNA that was separated was measured using Nanodrop 2000 (Thermo, USA). By calculating the absorbance ratio of 260/280 nm, the quality was examined. A score of 1.7 to 1.8 denotes high-quality DNA free of protein or RNA contamination.

DNA quality determination

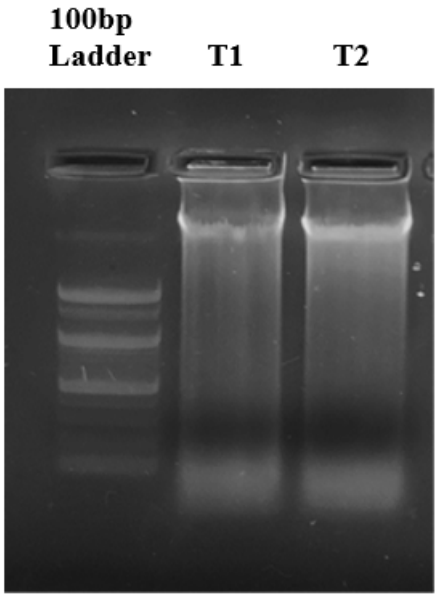

Fig.7: Genomic DNA

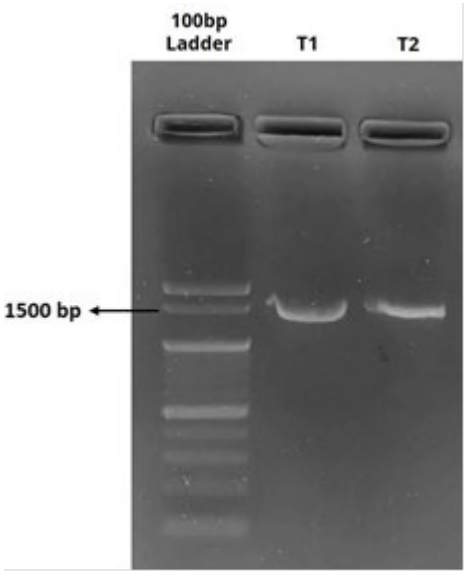

Fig.8: PCR products of 16s rRNA gene

On 0.8% agarose gels, the DNA quality was examined (Figs. 7 and 8). On 1.5%, the amplified products were examined. Using a molecular weight marker (a 100 bp ladder), the molecular weight was determined via agarose gel electrophoresis.

Chromium reduction potential

Redox processes can be catalyzed by microorganisms by a variety of methods, such as intracellular reduction and precipitation, nonmetabolic reduction by bacterial surfaces, and enzymatic extracellular reduction. Microorganisms capable of reducing Cr(VI) to Cr(III) include bacteria *(Desulfomamaculum, Achromobacter, Aeromonas, Bacillus, Enterobacter, Escherichia, Psuedomonas, Micrococcus)*, algae, yeasts, and fungi. (Fig 9) (Rahman Z and Thomas L 2021; Wang *et al.*, 2017).

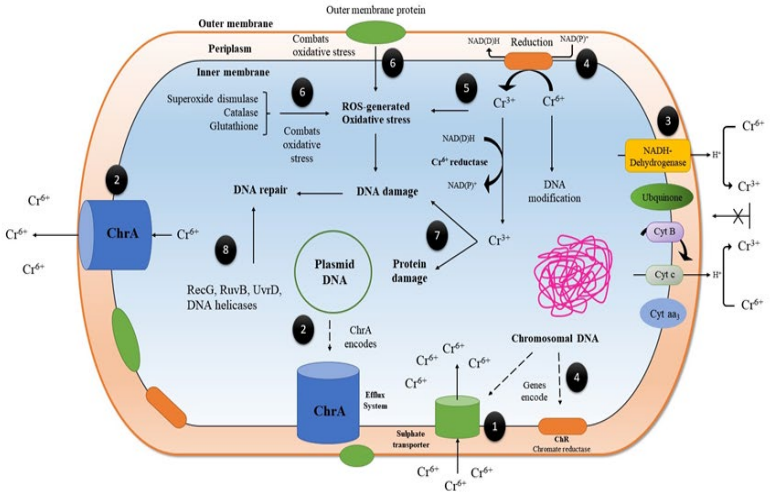

Fig 9: Intracellular Cr (VI) reduction mechanism

One frequent mechanism for reducing Cr(VI) intracellularly is the action of NADH, flavin protein, and other hemeproteins on the reduction of Cr(VI). Among these, related reductases function as intermediates and electron donors, respectively, as NADH and NADPH. The activity of chromate reductase in the cell-free extract of *Halomonas sp.* TA-04 can be significantly increased by NADH. In order for bacteria to reduce Cr(VI) intracellularly, they must first absorb Cr(VI) through the appropriate functional groups, then move Cr(VI) into the cell via sulfate or phosphate channels, reduce Cr(VI) intracellularly using certain pertinent substances, and finally immobilize and accumulate the reduced products of Cr(III). The plasmid has the ability to transfer Cr(III) products out of the cells, leaving a small amount of these compounds within the cell. Nonetheless, there have also been reports of the phenomena when decreased Cr(III) products precipitated and accumulated within the cell. When Cr(VI) is reduced, free radicals and reactive oxygen species (ROS) are produced, which can harm DNA under aerobic conditions. Furthermore, there is a harmful effect of decreasing Cr(III) on cells. An extracellular decrease is therefore preferable (Tahri Joutey *et al.*, 2016).

According to earlier studies carried out under microaerobic circumstances, chromium were precipitated from Cr (VI)-containing waters by two diverse mechanisms such as sulfate reduction and precipitation as sulfide. Sulfate reduction required fatty acids as organic substrates, which at first led to minimal sulfide accumulation. Based on unique morphological and biochemical traits, the chromium-resistant bacterial strain KK15—which was isolated from chromium-contaminated soil—was identified as *Pseudomonas olevorans*. The strain's hexavalent chromium resistance demonstrated its ability to withstand extremely high

concentrations of K_2CrO_4 in nutrient agar medium, and an assay involving crude cell-free extracts, resting cells, permeabilized cells, showed that the hexavalent chromium reduction is primarily linked to the soluble fraction of the cell. The strain holds great potential for the bioremediation of waste containing Cr(VI) (Smock *et al.,* 2020).

When treating industrial effluents in bulk using chemical procedures, the level is frequently not reduced enough to comply with environmental laws. Bioremediation is seen to be a superior option for pipe treatment at the end of the pipeline. There have been reports of heavy metal contamination at Kolkata's main sewage treatment plant, East Calcutta Wetlands, including chromium (VI). Thus, it's possible that the bacterial population in this area can withstand chromium (VI) and could be helpful for chromium (VI) bioremediation. A *Bacillus subtilis* strain that was obtained from this area was cultivated with chromium (VI) at concentrations ranging from 2.5 µg/L to 7.5 µg/L. After 24 hours, the residual chromium concentration in growth media decreased by 97% and 90%, respectively, from its starting values of 2.5 g/L and 5 g/L. The greatest removal was noted at 30°C. Using MATLAB® 7.4, non-linear regression analysis revealed that the *Bacillus* strain's growth in the presence of chromium (VI) provided the best fit for the Tessier model. The *Bacillus* strain may be able to remove chromium (VI) at the end of the pipe treatment (Upadhyay *et al.*, 2017). This suggests the potential statistical significance of chromium reduction using microbial strains.

Bravibacterium sp. CrT-11 to CrT-14 were among the bacterial strains that were isolated from tannery effluents. All of these strains were able to withstand very high concentrations of K_2CrO_4, up to 40 mg/ml on nutrient agar and 25 mg/ml in nutrient broth. They also showed resistance to several metals (Pb, Co, Cu, Mn, Zn, Ni etc) and antibiotics like streptomycin, ampicillin, tetracycline, kanamycin, chloramphenicol. Every strain exhibited the ability to aerobically convert Cr (VI) to Cr (III). Compared to the other strains, *Bravibacterium sp.* CrT-13 collects and reduces more Cr (VI) at all applied concentrations. Additionally, these bacterial strains absorb and degrade Cr (VI) found in industrial effluents; the addition of various metallic salts had no discernible effect on their ability to decrease Cr (VI) (Marzan *et al.*, 2017).

Elahi *et al.*, (2019) identified and characterized a multiple heavy-metal resistant hexavalent chromium-reducing *Microbacterium testaceum* strain B-HS2. The isolated strain is a promising option to restore Cr(VI) contaminated environments because to its strong capability for Cr(VI) reduction and multi-heavy metal tolerance. In 2019, Yuliani and colleagues identified and studied a chromium-lead resistant bacterium from the leather tanning sector in Malang, Indonesia (Yuliani *et al.*, 2019). The isolated strain was identified as Bacillus subtilis based on its morphological and biochemical features (Elahi *et al.,* 2019).

Microbial fuel cell performance

The viability of using the sMFC for soil bioremediation has been demonstrated by numerous investigations. According to these reports, the sMFC may have an impact on the remediation of soil heavy metal (loid) by: (1) modifying the soil chemistry considerably; (2) changing the amount of organic matter in the soil; and (3) changing the composition of the microbial community. The sMFC-induced alterations in the physical, chemical, biological characteristics of soil can have a significant impact on the transportation and destiny of certain redox-sensitive

heavy metal (loid) pollutants. The behavior of heavy metal (loid)s co-precipitated with mineral oxides is utilized as an illustration of the influence of the sMFC. Mineral oxides are used as a final electron acceptor by the extracellular respiring electrogenic bacteria in anaerobic environments when the anode is not present. As a result, the metal(loid) contaminants that co-precipitated with the mineral oxides dissolve and are released.

Microbial fuel cells (MFCs) have the ability to treat oxidized pollutants reductively through the use of their biocathode. In this work, we found putative Cr(VI) reducing microorganisms and showed reduction of biological Cr(VI) in an MFC cathode. The MFC's ability to generate power and reduce Cr(VI) was continuously observed. The only external carbon source introduced to the cathode compartment was bicarbonate, while the anode compartment received acetate as substrate. Microbial activity in the cathode, which uses electrons and protons created by the oxidation of acetate in the anode compartment, facilitated most of the Cr(VI) reduction. The minimal effect of abiotic processes and biomass degradation on Cr(VI) reduction served as evidence for this. The oxidation of acetate in the anode compartment produces protons and electrons that are used by microbial activity in the cathode to aid in Cr(VI) reduction. Abiotic processes and biomass decomposition have very little effect on the decrease of Cr(VI). The contribution of biomass decay and abiotic processes to Cr(VI) reduction was negligible. At starting Cr(VI) values less than 80 mg/L, a relatively quick Cr(VI) decrease was seen. Nevertheless, Cr(VI) reduction was incredibly sluggish at 80 mg/L. The highest Cr(VI) reduction rate of 0.46 mg Cr(VI)/g VSS· h was attained, leading to a corresponding increase in current (123.4 mA/m^2) and power density (55.5 mW/m2), respectively. The catholyte's supernatant contained no trace of reduced chromium, indicating total chromium elimination as $Cr(OH)_3$ precipitate. The cathode biomass was primarily dominated by phylotypes closely related to *Pseudomonas aeruginosa* and *Trichococcus pasteurii*, the potential Cr(VI) reducers, according to analysis of the 16S rRNA gene-based clone library (Yuliani *et al.*, 2019).

Hexavalent chromium can be easily reduced in the lab using a dual chamber MFC. Anolyte is generally the raw waste effluent and the bacteria acts like the substrate. In the present study *Bacillus australimaris* and *Bacillus albus* are used as substrates. Electrons are conducted in the anode which travels through the external circuit and enters the cathodic chamber. SPEEK as like proton membrane and reduces chromium which gets deposited on the cathode. Table 3 Depicts the performance of different MFCs in chromium reduction and energy generation studied in the previous years by many eminent scientists.

Table 3: Comparative data on previously studied MFCs that reduce chromium using microbes

Chromium initial concentration (mg/L)	**Source**	**Removal efficiency (chromium) (%)**	**Performance (mW/m2)**	**Reference**
100	Lysogenic broth with *E.coli*	100	1540	(Gupta, Yadav and Verma, 2017)

120	Lab inoculum	100	1221	(Li *et al.*, 2018)
204	Anaerobic sludge	99.5	1600	(Li, Zhang and Lei, 2008)
200	Domestic waste	100	150	(Wang, Huang and Zhang, 2008)
80	Mixed Anaerobic sludge	-	55.5	(Tandukar *et al.*, 2009)
-	Algal biomass	98	207	(P, 2013)
250	Anaerobic sludge	75	970	(Zhang *et al.*, 2012)
500	Anaerobic effluent	76	986	(Zhang *et al.*, 2012)
10	Sludge with *Hansenula anomala*	99.5	45	(Shi *et al.*, 2017)
30	Sludge with *Hansenula anomala*	99	45	(Shi *et al.*, 2017)
40	Sludge with glucose culture medium	100	164	(Song *et al.*, 2016)
5	Sewage sludge	22, 100	47	(Kim *et al.*, 2017)
10	Sewage sludge	19, 100	47	(Kim *et al.*, 2017)
30	Sewage sludge	16, 63	150	(Kim *et al.*, 2017)
50	Sewage sludge	23, 53	150	(Kim *et al.*, 2017)
20	*Shewanella oneidensis* MR-1	100	229	(Pang *et al.*, 2013)
5	Anaerobic sludge	Below detectable limits	92	(Sophia and Sai, 2016)
10	Anaerobic sludge	Below detectable limits	75	(Sophia and Sai, 2016)
5	Cow dung with lime	100	396	(Sindhuja *et al.*, 2018)
26	Cow dung with lime	100	397	(Sindhuja *et al.*, 2018)
10	*Shewanella oneidensis* MR-1	100	21	(Xafenias, Zhang and Banks, 2015)

100	Biofilm from anode	100	10	(Gangadharan, Nambi and Senthilnathan, 2015)
100	Anaerobic sludge	100	700	(Gangadharan, and Nambi, 2017)
20	Activated sludge with glucose medium	80	132	(Fei *et al.*, 2017)
81 ± 1.3	Tannery raw effluent with Nafion 117 as PEM	14.4	-	Present study
81 ± 1.3	Tannery raw effluent with SPEEK as PEM	51	-	Present study

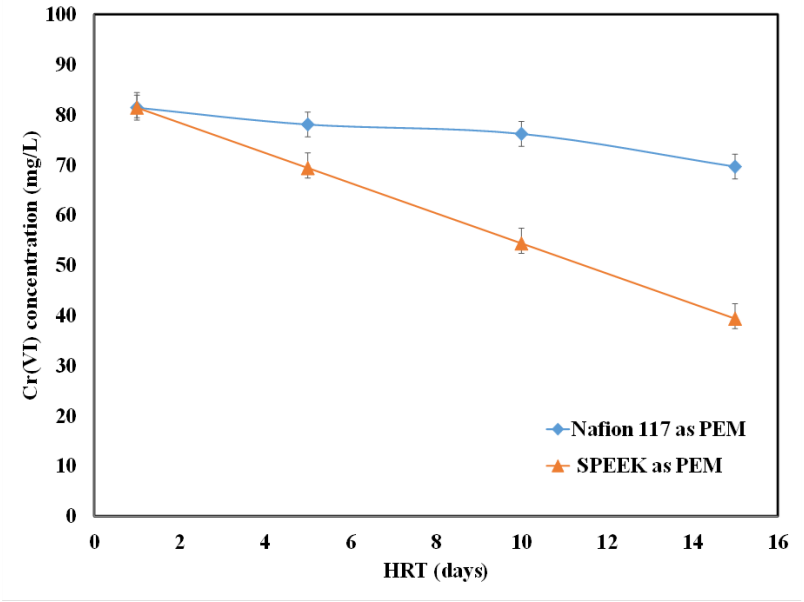

Fig 10. Biochemical reduction of Cr (VI) in both the reactors for HRT of 15 days at pH 2

Present studies have reported that a MFC with 500 ml working volume was analysed for chromium with Nifion 117 and SPEEK treated cathode. Figure 1 depicts the proposed mechanism of chromium reduction. When the circuit was completed chromium concentration was reduced by generating electrons. This reduction has been depicted in figure 10. Initial starting concentration was kept at 81 ± 1.3 mg/L. Nafion 117 treated MFC showed a gradual decrease in chromium concentration by 14.4% with a final value of 69 ± 2.5 mg/L post a fortnight. SPEEK treated MFC exhibited a reduction of 51% in chromium content with a final value of 39 ± 2 mg/L for the similar time period. Synergistic relationship between the microbial strains and SPEEK might be the reason for better chromium reduction in the second setup. SPEEK acts as a functionalized membrane for proton exchange and supports simple

protonation at the cathode. The presence of proton attracts the negatively charged $HCrO_4^-$ by electrostatic attraction. Thus leading to reduction of Cr(VI) to Cr(III). Although the Cr(VI) reduction is relatively lesser in the present study when compared to the previous studies (Table 3), the usage of low cost SPEEK as PEM, the easy availability of microbial consortia as inoculum and raw effluent as anolyte substrate, carbon paper as cheaper electrode make this a viable process for Cr(VI) reduction. However, the optimization studies to achieve complete Cr(VI) reduction and increased electricity production will be our priority for our forthcoming experimental studies.

LIMITATIONS

Currently experimental and optimization studies are being carried out in the laboratory using a 10 L dual-chambered MFC on a semi-batch mode. pH plays a major role in determining the activity of microbes, reaction rates, degradation of substrate and reduction of chromium at the respective compartments in the MFC. At lower pH the reduction seems to be better and as the pH increases the performance is found to decrease. Use of microbes in the anode chamber require neutral pH to enhance electron generation. Microbial growth and proliferation is majorly dependent on temperature. Depending on the species of microbes used temperature requirements vary. Optimal temperature required for microbial growth is around 25-35℃. Extreme conditions could harm the microbial components of MFC. In an ideal MFC setup, energy generation seems to be double at 40℃. But a further rise by 10℃ has reported a decrease in energy production by 4 times.

CONCLUSION

In the present study, bacteria were isolated and identified from leather tannery CETP, the isolated bacteria were identified to be *Bacillus australimaris* and *Bacillus albus* based on 16s rRNA sequencing. Niafion 117 treated MFC showed a gradual decrease in chromium concentration by 14.4% whereas SPEEK treated MFC exhibited a reduction of 51% for the similar time periods. Synergistic relationship between the microbial strains and SPEEK might be the reason for better chromium reduction in the second setup. Chromium-contaminated soils, tannery sludge, and effluents have yielded a variety of Cr-resistant bacteria, including *Bacillus species, Lactobacillus strains, Bacillus amyloliquefaciens, Bacillus cereus,* and *Bacillus methylotrophicus* strains. The future study will focus on culturing of the isolated strains *Bacillusalbus* and *Bacillus australimaris*. Upon obtaining successful results, the same will be scaled-up to 1000 litres pilot-scale reactor and filed studies will be conducted in the leather tannery industries for further collaboration and commercialisation.

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AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Vijay Samuel G, R Anitha, Nibedita Dey and

Govindarajan R. The first draft of the manuscript was written by D Sangeetha, Premkumar Thangavelu, M. Anthony Raj, A Saravanan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author Dr Nibedita Dey, upon reasonable request.

COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of Interest- The authors declare that they have no conflict of interest.

Ethical Approval- No ethical committee was required in the current study as it didn't deal with any animal or human samples.
Consent to Participate- No human or animal samples or organs were used for the current study
Consent to Publish- No human or animal samples or organs were used for the current study

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