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Biotreatment of Chromium Enriched Electroplating Effluent Using Bacterial Consortium

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ABSTRACT

The present is study focused on Lab-Scale biotreatment of chromium electroplating effluent using mixed bacterial strains. The electroplating effluent contaminated soil sample was collected and sixteen bacterial colonies were isolated and identified through morphological and biochemical characteristics. All the sixteen bacterial isolates were screened for metal tolerance using nutrient agar medium incorporated with chromium metal ions. Of the sixteen bacterial isolates, only four bacterial strains were found as potential metal tolerant bacterial strains and they were further characterized in the various environmental conditions such as different pH, various temperature and different chromium metal ion concentrations. The results of characterization study revealed that two bacterial strains, i.e., Pseudomonas sp 4 and Staphylococcus sp 2 were found to grow better in medium containing 300ppm of chromium in pH 7 at 37°C on 5th day. Based on molecular sequencing of 16S rRNA, the two bacterial isolates were confirmed asPseudomonas aeruginosa and Staphylococcus caprae. Antagonistic studies between two selected bacterial strains were performed and the results indicated with negative antagonistic activity between the strains. Therefore, these two compatible metal tolerant bacterial strains were further used as bacterial consortium for the treatment of chromium electroplating effluent in the Lab-scale reactor for 5 days. The treated effluent was collected and determined for various physicochemical characteristics through APHA, 1995 methods and also determined the presence of the residual metal through SEM-EDAX, FTIR and AAS analyses. The better performance metal removal was observed in the treatment with of bacterial consortium.

Key words: *Electroplating effluents, Chromium, Biotreatment, Bacterial consortium, Lab-scale bioreactor*

INTRODUCTION

In general, allindustrial developments result in generating wastewater in huge volume that contains high levels of conventional as well as non-conventional contaminants which affects aesthetic and the quality of water [1, 2]. The electroplating is one of the major industrial process consumes and discharges large volumes of hazardous, containing heavy metals as well as cyanides, hydrogen sulfides, ammonia and oil [3]. The major heavy metals used in the electroplating processes are copper, chromium, nickel, lead, cadmium, tin and zinc [4].

According to Jaishankar *et al*. (2014) and Nagajyoti *et al*. (2010), all of these heavy metals are considered to be significant environmental contaminants, and their toxicity is an issue that is becoming more and more important for ecological, evolutionary, nutritional, and environmental reasons [5, 6]. The heavy metals arsenic, cadmium, chromium, copper, lead, nickel, and zinc are the most frequently detected in waste water and are extremely dangerous to both human health and the environment [7]. More hazardous and linked to kidney impairment and lung cancer is chromium (VI). The US EPA regulates the discharge of Cr (VI) to surface waters and inland surface waters at a rate of less than 0.05 mg/L due to its toxic nature. In contrast, the total chromium, which includes Cr (III), Cr (VI), and other forms of chromium, is regulated to be discharged at a rate less than 2 mg/L [8].

However, diversed microorganisms including Bacteria are known to resistant heavy metals. Bacterial species possess the capacity to thrive in environments with elevated levels of metals and could also be crucial in the biological cycling of such metals, which holds immense promise for bioremediation [9, 10]. According to Dua *et al*. (2002), microbial bioremediation is a potential biological technique that is being extensively researched for wastewater treatment [11]. It uses microorganisms to break down or eliminate harmful waste components from the environment.

Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure [12, 13]. Therefore, the biological processes are consider to be an environmental friendly, cost-effective and enable to reduce the operating costs and chemicals' requirement for treatment. It is also effective to be applied at lower levels of contamination and act as an alternative for conventional treatments [14, 15]. In view of the above reason, the present study is focused on "study on lab-scale biotreatment of chromium enriched electroplating effluent using indigenous bacterial consortium".

MATERIALS AND METHODS

Samples collection

The electroplating effluent sample used for the study was collected fromdirect outlet of Vishnu Velava Bright Industry, Madurai, Tamil Nadu. The electroplating effluentcontaminated soil samples for screening of metal tolerant bacterial strains were collected from the contaminated sites near Vishnu Velava Bright Industry, Madurai. Both effluent and soil samples weretransported to the laboratory, Department of Biology, The Gandhigram Rural Institute - Deemed University, Gandhigram for further analysis.

Screening of selected bacterial isolated for chromium resistance

The sixteen predominant bacterial strains were isolated from the electroplating effluent contaminated soil samples and were screened for its potential to tolerate chromium metal using standard procedures [16, 17]. Sixteen selected bacterial strains were inoculated on nutrient agar medium incorporated with chromium metal (100ppm) and incubated at 37°C at 5 days. The comparative growth performance of all bacterial isolates were observed and recorded.

Characterization of four selected heavy metal tolerant bacterial isolates

Four selected metal tolerant bacterial strains were characterized with different conditions using standard procedures [18]. Bacillus sp. 1, Escherichia coli, Pseudomonas sp. 4, and Staphylococcus sp. 2 are four potential metal-tolerant bacterial strains that were characterized by cultivating them in a metal-based nutrient agar medium under a range of environmental conditions. These conditions included pH levels (5, 7, and 9), temperature ranges (5°C, 28°C, 37°C, and 45°C), and differing concentrations of Cr (VI) metal (100, 200, 300, and 400 ppm) in various treatments for a period of five days. The growth performance and its tolerance to chromium metal in four bacterial isolates were measured by optical density at 540 nm.

Molecular identification of metal tolerant bacterial isolates

The two predominant metal tolerant bacterial strains were identified through molecular sequencing using standard procedures [19]. The genomic DNA was extracted from two potential strains (*Pseudomonas* sp 4 and *Staphylococcus* sp 2) by using INSTA GENE™ MATRIX GENOMIC DNA ISOLATION KIT. 16S rRNA GENE amplification was carried out and it provides 1510 base pair product for *Pseudomonas* sp 4 and 1508 base pair product for *Staphylococcus* sp 2. Further, the sequences were processed for trimming at both 3' and 5' ends. The software "Biosystem ABI 3730xl sequencer" was used to process bacterial sequencing data. Online

tool Blast N available at the National Center for Bioinformatics (NCBI), USA, was used to comparethe 16S rRNA sequence of *Pseudomonas* sp 4 and *Staphylococcus* sp 2 with standard NCBInucleotide database, followed by the bacterial sequence data was aligned and analyzed for identifying the organisms. The NCBI Blast sequenced database for *Pseudomonas* sp 4 and *Staphylococcus* sp 2showed 86% query similar with *Pseudomonas aeroginosa* and 95% similar with *Staphylococcus caprae* respectively.

Study on antagonistic activity between two metal tolerant bacterial strains

The Antagonistic effects between two metal tolerant bacterial strains *P. aeruginosa* and *S. caprae*were tested using standard method [20]. A loopful of bacterial culture was taken and parallely streaked on nutrient agar medium containing petridish. The plate was incubated at 37°C for 24 hrs and the results were recorded.

Preparation of bacterial consortium

Based on the negative antagonistic effects, the two potential metal tolerant bacterial strains viz., *P. aeruginosa* and *S. caprae* were selected and mass cultured individually. Then, cultures of two bacterial strains were mixed together in 1:1 ratio carrying a fixed cell density of 1×10^6 cell/ml and used for the biotreatment process of chromium effluent.

Lab-scale biotreatment study on chromium effluent using mixed bacterial isolates

Biotreatment process of chromium effluent was studied with mixed culture of two potential metal tolerant bacterial isolates, *P. aeruginosa* and *S. caprae* in glass column reactor using standard procedure [21]. Effluent based nutrient broth (500ml) was prepared and sterilized at 121°C at 15lbs for 20 mins. The sterile media was transferred to 1 liter glass column and inoculated with microbial consortium of two metal tolerant bacterial isolates (5ml/500ml media with cell density of 1×10^6 cells /ml^{-1}). The bacterial reactor was run for 5 days. The bacterial metal effluent was collected after 5 days and determined the physicochemical characteristics [22], SEM- EDAX [23], FTIR [24] and AAS [25] analyses and the results were recorded.

RESULTS AND DISCUSSION

Screening of selected bacterial isolates for chromium metal tolerance

Using morphological and biochemical traits, sixteen dominant bacterial colonies were isolated and identified. Subsequently, the genus of bacteria was determined by contrasting the features of several isolates with Bergy's Manual of Determinative Bacteriology [26]. Utilizing nutrient agar medium supplemented with 100 ppm of chromium metal ions, all sixteen of the bacterial isolates were tested for their ability to withstand chromium metal. Based on the growth performance, only four bacterial strains like *Bacillus* sp 1, *E. coli*, *Pseudomonas* sp 4 and *Staphylococcus* sp 2 shown better growth compare were selected for further study **(Table 1)**.

Several authors have already reported bacterial strains capable of metal tolerant and particularly bacterial strains such as *Bacillus* sp, *E. coli*, *Pseudomonas* sp and *Staphylococcus* sp, *Salmonella* sp and *Shigella* sp were found to potential metal tolerant strains [27-29].

Table 1. Characteristics of sixteen bacterial isolates screened for chromium metal tolerance in culture medium with 100ppm Cr (VI)

Bacterial Isolate No.	Bacterial Strain	Growth Performance Scale				
		Day 1	Day 2	Day 3	Day 4	Day 5
BIS-1	*Pseudomonas* sp 1	PG	MG	GG	EG	EG
BIS-2	*Proteus* sp 1	NG	PG	MG	GG	EG
BIS-3	*Shigella* sp 1	NG	NG	PG	MG	EG
BIS-4	*Escherichia coli*	MG	GG	EG	EG	EG
BIS-5	*Proteus* sp 2	NG	PG	MG	GG	EG
BIS-6	*Pseudomonas* sp 2	NG	PG	MG	GG	GG
BIS-7	*Pseudomonas* sp 3	NG	NG	PG	MG	GG
BIS-8	*Shigella* sp 2	PG	MG	GG	EG	EG

BIS-9	*Salmonella* sp 1	PG	MG	GG	EG	EG
BIS-10	*Salmonella* sp 2	NG	PG	MG	GG	EG
BIS-11	*Pseudomonas* sp 4	MG	GG	EG	EG	EG
BIS-12	*Micrococcos* sp	NG	PG	MG	PG	EG
BIS-13	*Bacillus* sp 1	MG	GG	GG	EG	EG
BIS-14	*Staphylococcus* sp 1	NG	NG	MG	GG	EG
BIS-15	*Staphylococcus* sp 2	GG	EG	EG	EG	EG
BIS-16	*Bacillus* sp 2	NG	PG	MG	GG	EG

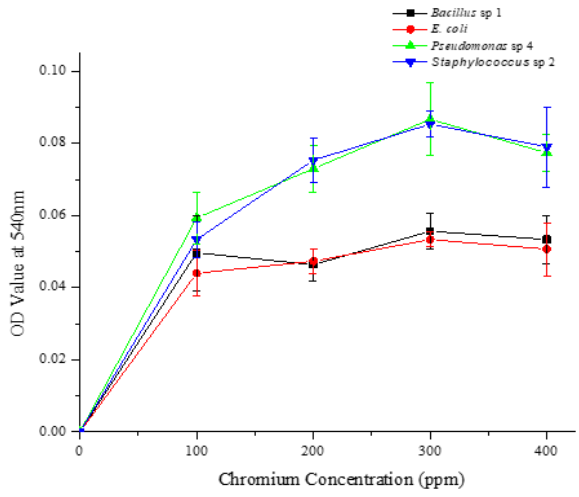
NG: No Growth**; PG:** Poor Growth**; MG:** Moderate Growth**; GG:** Good Growth**; EG:** Excellent Growth

Characterization of four selected chromium tolerant bacterial isolates

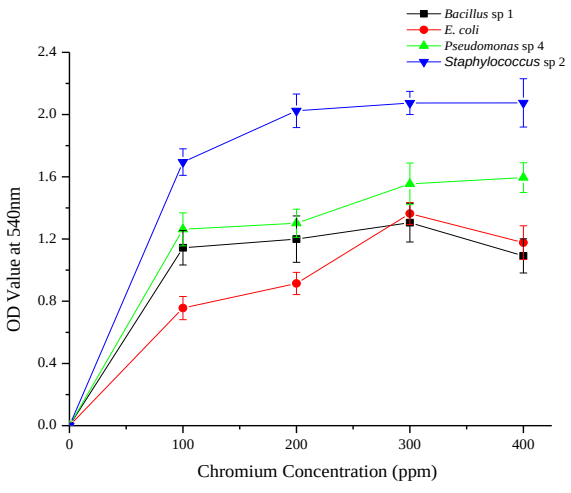
Characterizedfour potential chromium tolerant bacterial strains, *Bacillus*sp1, *E. coli*, *Pseudomonas* sp 4and*Staphylococcus* sp 2 with various growth conditions such as pH, temperature and substrate concentrations and the experimental results reveals that two bacterial strains i.e., *Pseudomonas* sp4and *Staphylococcus* sp 2 grown well on 400ppm of chromium containing nutrient broth medium with pH 7 at 37°C **(Figures 1-3)**. These two bacterial strains have capacity to tolerate higher concentration of chromium in log phase.

Six possible chrome metal resistant bacterial strains have already been reported by Ranjithkumar and Mahalingam (2016) [30]. These strains were identified by cultivating them in nutrient agar medium based on chromium under varied environmental conditions. In a similar vein, bacterial strains that were discovered and identified by Nihar and Varsha (2010) demonstrated chromium resistance [27]. The ability of the bacterial strains to withstand and grow at various chromium (VI) concentrations was also reported by Seema *et al*. (2012) [31]. The bacterial strains showed optimal growth at high chromium (VI) concentrations, which they can tolerate up to 500 mg/L.

In another study, sixty-eight morphologically distinct Cr^{6+} resistant bacterial strains were isolated and their tolerance limit was determined. Of the 66 strains only four isolates have been found potential tolerant to elevated chromium concentration [32]. In a previous study, Micrococcus sp. at pH 7.0 removed 90% of the chromium. Additionally, it has been found that B. licheniformis bioaccumulated lead at a rate of 0 to 1.1 mol metal/g biomass. Tripathi (2011) reported that B. cereus was able to bioremediate 74.5% Cr (VI) in 48 hours.



a)



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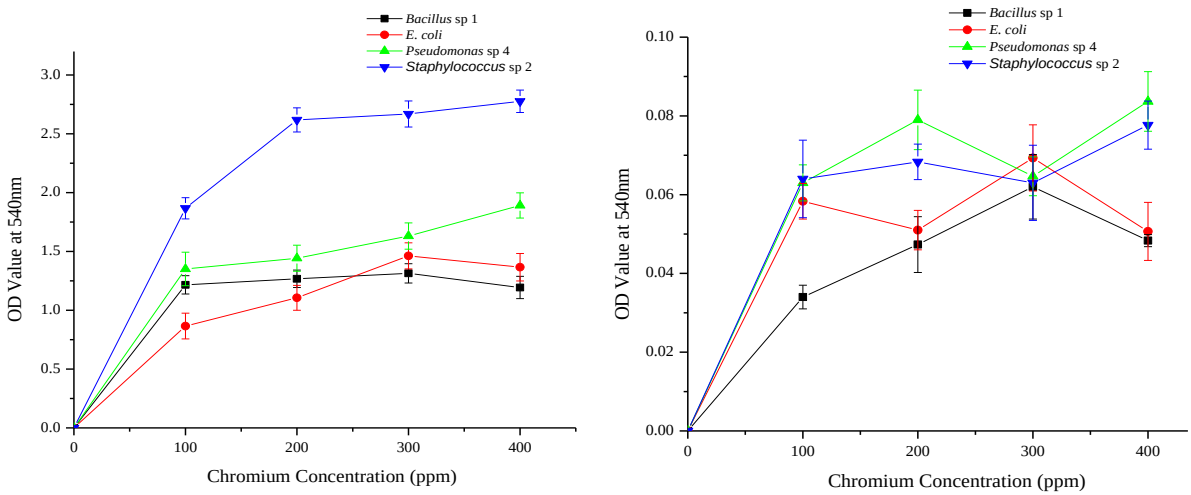


Figure 1. Growth performance of four different bacterial strains in nutrient broth supplemented with different concentrations of chromium (100ppm, 200ppm, 300ppm & 400ppm) in pH 5 at various temperatures (5°C, 28°C, 37°C & 45°C) on 5th day. a) At 5°C. b) At 28°C. c) At 37°C. d) At 45°C

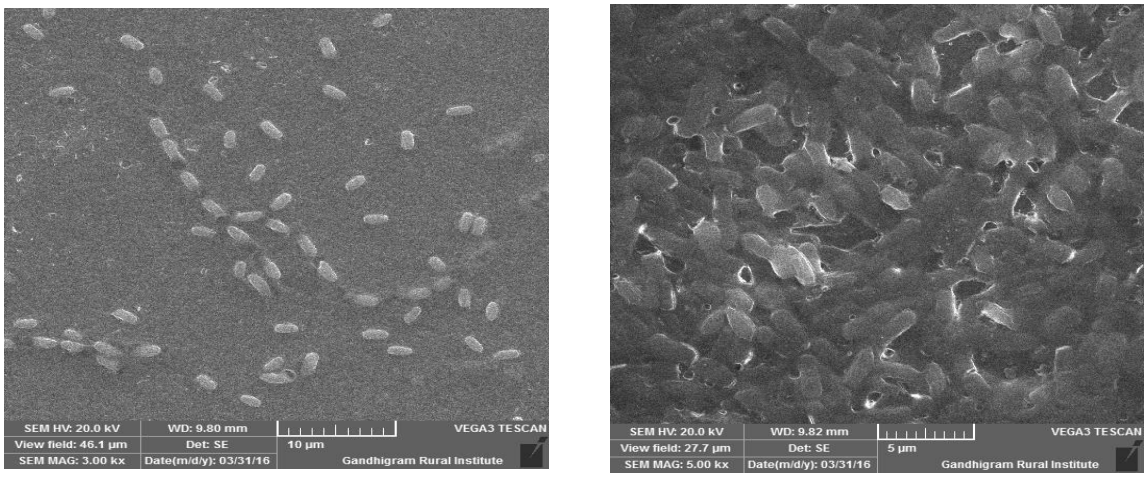


Figure 2. SEM image of chromium tolerant bacterial strains. a) *Pseudomonasaeruginosa.* b) *Staphylococcuscaprae*

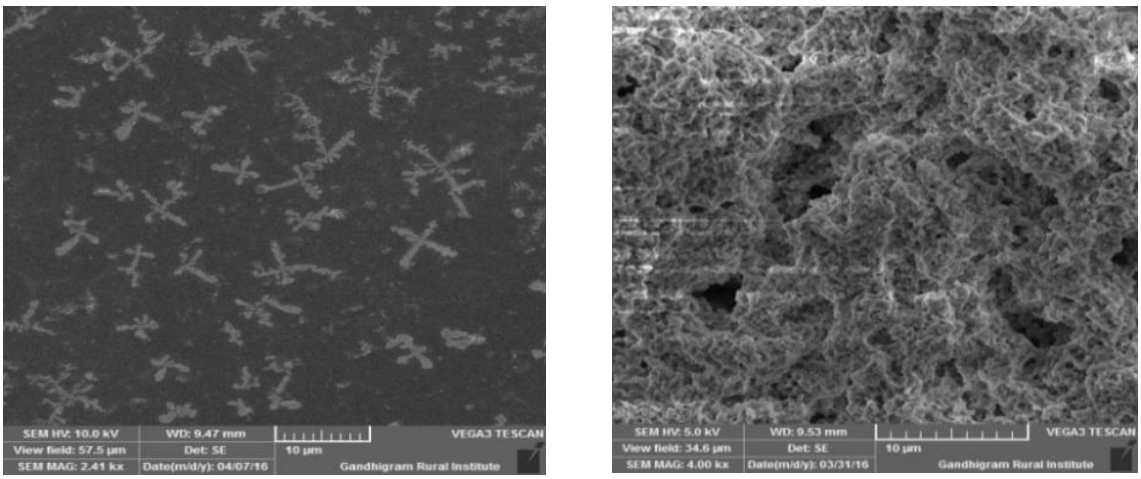


Figure 3. SEM image of chromium electroplating effluent. a) Untreated effluent. b) Bacterial treated effluent

Study on antagonistic activity between two chromium tolerant bacterial isolates

The Antagonistic effects between two chromium metal tolerant bacterial strains were tested and the results revealed that, the zone of inhibition not found between *P.aeroginosa* and *S. caprae*. Elhartit *et al.* (2015) also reported on studied the antagonistic activity through zone of inhibition between the different isolates of Bacillaceae and Pseudomonadaseae family against pathogenic fungi [33, 34].

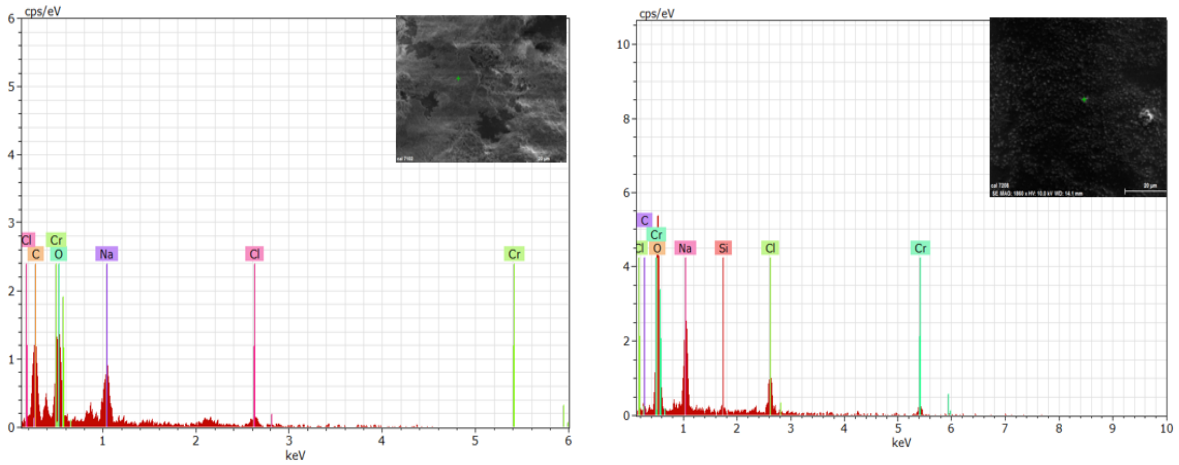
Lab-Scale biotreatment study on chromium effluent using mixed bacterial isolates

Biotreatment process of Chromium effluent was studied with mixed culture of two potential metal tolerant bacterial isolates, *P.aeroginosa* and *S. caprae*in Lab-scale glass column reactor. The bacterial metal effluent was collected after 5 days and determined the physicochemical characteristics, SEM-EDX, FTIR and AAS analyses **(Table 2, Figures 4 and 5)**.

Table 2. Physico-chemical characteristics of chromium electroplating effluentbefore and after the bacterial treatment

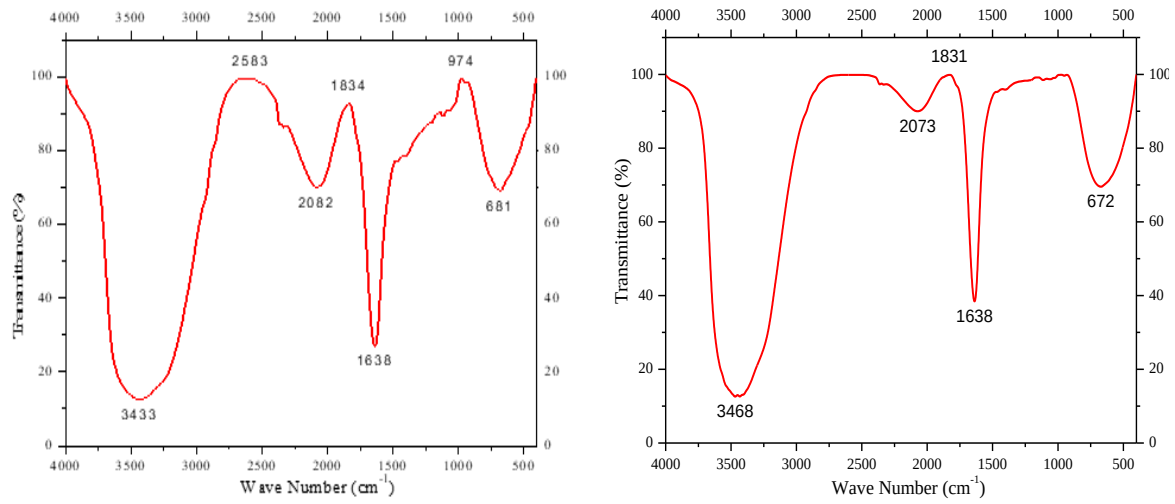
S.No	Physico-Chemical Parameters	Untreated			Bacterial Treated		
1.	Temperature (˚C)	31.3	±	0.9	30.5	±	0.6
2.	pH	3.7	±	0.3	31.2	±	3.0
3.	Total Suspended solids (mg/l)	1047. 7	±	10.6	1028	±	5.8
4.	Total dissolved solids (mg/l)	8663.3	±	11.6	11901	±	28.1
5.	Hardness(mg/l)	18.4	±	0.6	10638	±	13.1
6.	Chloride(mg/l)	266.7	±	0.4	15.3	±	3.8
7.	Calcium(mg/l)	126.1	±	0.9	11.25	±	0.6
8.	Sodium(mg/l)	148.6	±	0.5	24.9	±	0.5
9.	Potassium(mg/l)	218.3	±	0.6	42.2	±	10.3
10.	Dissolved oxygen(mg/l)	95.3	±	8.0	1783.3	±	8.3
11.	Biological oxygen demand (mg/l)	410.3	±	8.5	66.3	±	6.4
12.	Chemical oxygen demand (mg/l)	1066.0	±	10.8	420.2	±	13.2
13.	Chromium (ppm) as per AAS	17.5326	±	0.3677	13.5564	±	0.0007

(Values are mean of three replicates ± standard error)



a) b)

Figure 4. EDAX image of chromium effluent using bacterial consortium. a) Untreated effluent. b) Bacterial trea effluent



a) b)

Figure 5. FT-IR Spectra of chromium electroplating effluent sample. a) Untreated effluent. b) Bacterial treated effluent

In this study, SEM and Energy Dispersive X-ray analysis (EDX) were used to quantify the amount of chromium present in the bacterial strains *P. aeroginosa* and *S. caprae* as well as to evaluate morphological alterations in response to chromium accumulation. After five days of incubation without exposure to chromium, SEM analyses of two bacterial strains, *P. aeroginosa* and S. caprae, were displayed **(Figure 4)**.

These results are in agreement with SEM analysis of Cr (VI) treated with *P.aeroginosa*cell by Suparna *et al.* (2011) [35]. Their findings from SEM investigations show that prior to Cr (VI) biosorption, the cells seemed plump with smooth surfaces in a loosely-bound state. P. aeruginosa treated with Cr (VI) develops a rough, protruding, and uneven cell surface. Since X-ray absorption provides information on the electronic and structural state of an element, EDX was used to confirm the sorption products on the surface of the bacterial cell.

Ramrakhiani *et al*. (2011) recently reported on their SEM-EDX analysis of Cr (VI) biosorption [36]. They collected metal SEM micrographs and EDX spectra for the metals both before and after biosorption onto the biomass. The micrographs showed that the surface of T. clypeatus cells loaded with metal clearly showed the existence of new shiny bulky particles. EDX analysis, which identified each metal peak(s) in the spectra, further supported this observation [37].

In this study, the results of FTIR spectra before and after Cr (VI) bioremediation are shown in **Figure 5** were the spectra range of 4000–400cm^{-1} is to determine the interaction of the metal ions and to identify functional groups which are responsible for the Cr (VI) bioremediation process. Broad spectra bands were observed at 3433cm^{-1}and 3468cm^{-1}indicating the presence of N-H stretch. The N=C=S stretching and the C=C=Sstretching were observed at 2082cm^{-1} and 2073cm^{-1} respectively. The changes in peaks observed between 1831cm^{-1} and 1638cm^{-1} could be due to the bending vibration of C-H group C=C in the remediation of Cr (VI). The band at 681.76cm^{-1}was shifted to 671.91cm^{-1}on Cr (VI) loaded biomass and corresponds to the stretching bond of the C =O group. Another change in the spectrum 2583cm^{-1} was not obtained after Cr (VI) bioremediation. In this study, the N-H stretching group is mainly involved in Cr (VI) metal bioremediation. Peaks in the region of lower wave numbers (<800 cm−1), can be assigned to bending of aromatic compounds.

Further the concentration of chromium in electroplating effluent was determined before and after the bacterial treatment by an Atomic Absorption Spectrophotometer(AAS) with flame atomization. The results revealed that concentration of chromium was decreased from 17.7326ppm to 17.7092ppm **(Table 2)**. Similar study was undertaken by Konstantinos *et al.* (2011) and they were noted thatthe metal uptake was determined based on the difference between the primary and secondary concentrations [25]. Further, it was also noted in different studies, that the uptakes of Cd optimum 87 mg/g for *Sargassum vulgare*, 80 mg Cd/g for *S. fluitans*, and 74 mg/g for *S. filipendula*. Uptakes of Cu at pH 4.5 were qmax = 59 mg/g for *S. vulgare* 56 mgCu/g for *S. filipendula* and 51 mg Cu/g for *S. fluitans* [38]. Also, Kaewchai and Prasertsan (2002) studied the Ni and Cd adsorption by dried cells of *E. agglomerans* SM 38 and found that at optimum pH their removal reached 25.2% and 32%, respectively [39].

Based on this study, it was suggested that the two metal tolerant bacterial strains, *P. aeruginosa* and *S. caprae* would be used as bacterial consortium for bioremediation studies.

CONCLUSION

The extensive study on Lab-scale biotreatment of Chromium enriched electroplating effluent using bacterial consortiumresults in identification of two potentialCr^{6+} tolerant bacterial strains and would used as effective bacterial consortium for the biotreatment of chromium metal contaminated effluents before discharging into the open filed so as so to conserve the environment and the nature.

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ETHICS STATEMENT : None

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