J. Appl. Sci. Innov. Technol. 4 (2), 64-78 (2025) ISSN (Online): 2583-505X

PMSST

Journal of Applied Science, Innovation & Technology (JASIT)

Journal homepage: https://prakritimitrango.com/documents-reports-and-publications/journal/

Research article

Reduction of hexavalent chromium by *Micrococcus endophyticus* isolated from leather industry wastewater

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ARTICLE INFOR

Article history:
Received: 06 August 2025
Revised: 30 September 2025
Accepted: 04 October 2025
Published online: 31 December 2025

Keywords:
Leather industry wastewater
Chromium reduction
Micrococcus endophyticus
SEM analysis
FT-IR analysis

ABSTRACT

The study aimed to isolate and characterize bacteria capable of reducing hexavalent chromium (Cr^{6+}) present in wastewater discharged from the leather industry (LIs).The bacterial isolate AAKC5 was successfully obtained and characterized using 16S rRNA sequencing. The isolate demonstrated a tolerance capacity of up to 2000 mg/L Cr^{6+}.Phylogenetic analysis of the 16S rRNA gene sequence identified the strain as *Micrococcus endophyticus* (PP830646). The study presents a novel finding wherein isolated bacterium demonstrated the ability to reduce Cr^{6+} at greater concentrations higher than previously reported. In experiment involving Cr^{6+}reduction across varying concentrations ranging from (250-2000) mg/L, the bacterium exhibited notable reduction efficiencies ranging from 89.9% (250 mg/L) to 81.02% (2000 mg/L) over a period of 96-120 hours. Scanning electron microscope (SEM) analysis comparing control cells with those exposed to 2000 mg/L Cr^{6+}revealed an increase in cell size, which was likely due to adsorption or precipitation of reduced Cr^{3+} on the bacterial surface. Fourier transform infrared spectroscopy (FT-IR) spectroscopy further indicated that biomass carboxylate and amino conjugates played a key role in the bio reduction of Cr^{6+}.Additionally, Gas chromatography mass spectrometry (GC-MS) analysis of untreated and *Micrococcus endophyticus* (AAKC5)treated leather industry wastewater (LIWW) showed a significant reduction in toxic compounds, demonstrating effective microbial bioremediation. Overall, the study showed that an isolated strain of *Micrococcus endophyticus* effectively reduced Cr^{6+}present in LIssediments and could be applied for the detoxification of heavy metal contaminated leather effluent sites.

1. Introduction

The ecology and public health were increasingly threatened by the contamination of natural habitats such as water and soil with harmful metals, necessitating suitable corrective measures. Heavy metals including Chromium (Cr), Cadmium (Cd), Mercury (Hg), Arsenic (As), Lead (Pb), exerted hazardous effects on both the environment and human health,makingthecritical pollutant of concern(Saran et al., 2025a).In developing nations, various formsof industrial waste (liquid and solid) with high concentrations of hazardous metals were indirectly or directly released without treatment (Saran et al., 2024a).

The major industries that cause toxic metal contamination include metallurgy, pigments, dyes, leather, wood preservation, and refractory brick production (Kashyap et al., 2023;Devi et al., 2022).Among these, the tannery sector was recognized as

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the prime contributor to environmental Cr pollution. Since tanning wasentirelyawet process, LIs consumed large volume of water and generated 30 to 35 L of effluent per kilogram of processed hides or skin (Saran et al., 2023; Devi et al., 2023a).India housed~2,091 tanneries, focused mainly in West Bengal, Bihar, Gujarat, Uttar Pradesh, Andhra Pradesh, Maharashtra, and Tamil Nadu, which collectively discharged nearly 175,000 cubic meters of wastewater each day (Devi et al., 2025a).In Uttar Pradesh, about 444 operational tanneries, primarily located in Unnao and Kanpur, produce 22.1 MLD of effluent daily (CPCB, 2013). This wastewater contained Cr^{6+}concentration ranging from 0.01 to 4.24 mg/L (MOWR 2013). Nearly 80% of the LIs employed Cr based tanning processes, leading to the annual release of 2000-3200 ton of Cr into the environment. Leather effluents contained 2000-5000 mg/L of Cr, far exceeding the permissible limit 2 mg/L for wastewater discharge. Unlike organic pollutants, heavy metals did not degrade but accumulated in the environment, eventually entering the food chain and exerting, mutagenic, carcinogenic, and genotoxic effects (Devi et al., 2025a).

Cr was widely used in several industries owing to its extensive application in steel, paper, automobile factories, electroplating paint coating machine, tanning agent, mineral ore processing (Khanam et al., 2024). In the natural environment, chromium commonly existed in two stable oxidation states: Cr^{3+} and Cr^{6+}. Cr^{3+} functioned as an essential micronutrient and was comparatively nontoxic. Appropriate levels of Cr^{3+}were essential to human health, particularly in lipid and glucose metabolism. The USEPA mandated a permissible Cr concentration of 0.1 mg/L in drinking water (Akhzari et al., 2024).

The World Health Organization (WHO) recommended that drinking water should not contain more than 0.05 mg/L of Cr. In contrast, Cr^{6+} was recognized as a highly dangerous oxoanionic species with severe environment and health impact, even at low concentrations. Due to its high solubility and toxicity, Cr^{6+} readily crossed cell membranes and interacted with nucleic acids and proteins. In humans, the accumulations of Cr^{6+}resulted in serious health issues, including mental retardation, renal failure, cancer, and other problems (Patilet al., 2025).

Cr^{6+} was reduced through the reduction process to the less hazardous Cr^{3+}form, Cr reducing bacteria were microorganisms capable of $Cr^{6+}$to Cr^{3+}(Tan et al., 2020). Traditional Cr^{6+} removal methods, such as photocatalytic reduction, were unsuitable for large scale application due to high costs and secondary pollutant generation (Saran et al., 2025b). Moreover, chemical methods were ineffective at low concentrations. Thus, reducing Cr^{6+}using Cr reducing bacteria proved to be an efficient and ecologically beneficial strategy (Devi et al., 2025b).

Algae have been recognized as the most common microorganisms used for biosorption because their cell walls provide exceptional ability to bind metals (Devi et al., 2023b; Saran et al., 2024b; Saran et al. 2024c). In addition, fungi, and algae, owing to their diverse metabolic capacities, strong shape, and cell wall fraction, served as effective biosorbents (Satpati et al., 2024; Sharma et al., 2021). However, while fungi and algae received comparatively less attention in bio reduction processes, bacteria were predominantly employed in most earlier studies for the reduction of Cr^{6+}(Ismail and Moustafa, 2016;Devi et al., 2024).These observations highlighted that an essential step in removing $Cr^{6+}$from leather industry wastewater (LIWW) was the screening and selection of suitable bacterial strains (Devi et al., 2023a).Microbial remediation was considered the most efficient and cost-effective approach to address Cr pollution (Sanjay et al., 2020). The reduction of Cr^{6+} into Cr^{3+}remained the most widely used strategy (Sophia and Saikant, 2016). Nonetheless, the limited availability of appropriate microbial resources restricted the effective application of in situ bioremediation (Lin et al., 2023).

Temperature, nutrition availability, and strain type were critical factors influencing Cr^{6+}bioremediation (Tan et al., 2020). Thus, selecting the most efficient strain was essential for effective Cr^{6+}reduction into Cr^{3+}. In this study, five strains were isolated from LIWW, among which AAKC5 showed the highest Cr^{6+}reduction efficiency and was identified as *Micrococcus endophyticus.* The role of its biogenic extracellular polymeric substances (EPS) in Cr^{6+} reduction was evaluated at different concentrations, marking the first use of *Micrococcus endophyticus* for Cr^{6+} bio reduction. There were five recognized species in the genus *Micrococcus*: *M. lylae*, *M. endophyticus*, *M. luteus*, *M. antarcticus*, and *M. flavus*. Within this genus *M.luteus* (NCTC2665, DSM 20030T, "Fleming strain") served as the types pieces and possessed one of the shortest genomes ever sequenced among actinobacteria.

This research aimed to identify species of *M. endophyticus* capable of bioremediation of Cr^{6+} contaminated wastewater. After preliminary screening, molecular characterization confirmed the isolate as *Micrococcus endophyticus*. Unlike previous studies that primarily focused on the well-documented *M. luteus* or other microbial species for Cr reduction, this study was the first to report the bio reduction potential of *M. endophyticus*. The work established its novelty by demonstrating not only its tolerance to high Cr^{6+} concentration but also its efficiency in reducing the metal, thereby highlighting its promise as a new microbial resource for sustainable bioremediation of Cr polluted effluents.

2. Materials and methodologies

2.1. Collection of leather industry wastewater

LIWWwas collected in pre-sterilized 10-L jerry cans from the Common Effluent Treatment Plant (CETP),Unnao Tanneries Pollution Control Company, situated in A-7, Site-II UPSIDC Industrial Area, Banthar, Unnao, India (26°29'09.6"N 80°27'38.3"E). Samples were transported to the laboratory in an iceboxwithin 4-6 hours, immediatelyprocessed upon arrival, and stored at 4^{o}C until further use.

2.2. Physico-chemical analysis of leather industry wastewater

Following the standard procedures for examining wastewater and water, in triplicate physico-chemical examination of the wastewater from leather industry was conducted. The collected LIWW, analysed for Colour, Odour, pH, Temperature (°C), Conductivity (mS/ mg/L), BOD (Winkler method), COD (Dichromate mercury free method), Total dissolve solids (TDS), Chloride ($AgNO_3$ titration method), Total hardness (EDTA titrimetric method), Chromium (Di-Phenyl Carbazide) methods(Lipps et al., 2023).

The physico-chemical analysis of wastewater was carried out following the methods of Baird et al. (2017). Colourwas graded on a scale of 0 (clear) to 70 colour units, with pure water assigned a value of 0. Theodour of water originated from organic matter, inorganic chemicals, or dissolved gases, which could be derived from residential, agricultural, or natural sources. The pH, temperature, and electrical conductivity (EC) of wastewater samples were measured *in situ* using a glass electrode pH meter, a mercury thermometer, and a conductivity meter respectively. Biochemical Oxygen Demand (BOD_5) was determined by the standard five-day dilution method. Chemical Oxygen Demand (COD) was analysed using strong oxidising chemicals (Potassium dichromate), sulphuric acid, and heat. Total dissolved solids (TDS) were measured using a TDS metre. Chloride concentration was estimated by silver nitrate titration with potassium chromate indicator, while total hardness was determined by EDTA titration using Eriochrome Black T indicator.

2.2.1. Analysis of Total Chromium metal in untreated and treated LIWW

The concentration of total chromium in wastewater samples collected from LIs was determined following standard analytical procedures. Aciddigestion followed by Atomic Absorption Spectrophotometer (AAS) (VARIAN AS240FS, Australia) was performed as described by Lipps et al. (2023). Fordigestion, 100 mL of filter-sterilized wastewater was mixed with concentrated nitric acid (6 mL) and perchloric acid (1 mL) in a 6:1 ratio. The mixture was heated on a hot plate under a watch glass until the solution cleared and yellow fumes appeared. After cooling, the digested sample was filtered through Whatman No. 44paper, diluted to 10 mL with deionized water, and analysed using standard metal solutions (Saran et al., 2025a).

2.2.2. Analysis of detoxification of Cr^{6+} by using DPC

Cr reduction was analysed using the DPC (dipheneylcarbazide) procedure i.e., "DPC reagent was prepared by dissolving 250 mg of DPC in 50 mL of acetone. The reaction mixture contained 100 or 200 µL of culture supernatant, 330 µLof 6 M H_2SO_4, 400 µL of DPC reagent and the final volume was achieved to 10 mL using distilled water". The reaction mixture was kept at room temperature for 20 min and the absorbance of the resulting colour complexwas measured at 560 nm (Sanchez-Hachair and Hofmann, 2018;Thacker and Madamwar, 2005).

2.3. Isolation and screening of chromium resistant bacterial strain

Wastewater from LIs was collected, serially diluted, and spread onto Luria-Bertani (LB) agar plates amended with potassium dichromate ($K_2Cr_2O_7$) at concentration ranging from 250–2000 mg/L.The plates were incubated at 35±2 °C for 24-48 hours to isolate Cr resistant bacteria (Khanam et al., 2024; Farag and Zaki, 2010). Colonies showed diverse morphologies were subsequently transferred to LB agar plates containing increasing concentrations (250–2000 mg/L) of $K_2Cr_2O_7$ to assess maximum Cr^{6+} tolerance. Among five isolates, only three strains (AAKC1, AAKC3, and AAKC5) tolerated 2000 mg/L Cr^{6+}. These strains were selected for further biochemical characterization and identification through 16S rRNA gene sequencing to confirm their taxonomic status and evaluates their potential for Cr bioremediation.

2.4. Characterization and identification of isolated bacteria

2.4.1. Characterization of isolated bacteria on the basis of morphological and biochemical

The isolated bacterium was classified based on its morphological and biochemical characteristics, following the protocols outlined in Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham, 1993). Subsequently, its identification was confirmed through 16S rRNA gene sequencing analysis.

2.4.2. Analysis of the 16S rRNA gene sequencing with gene-bank accession number

Genomic DNA was extracted from an overnight bacterial culture using the alkaline lysis method. A 1217 bp fragment was amplified with 5 µL of template DNA and universal 16S primers (forward: 5'-GGATGAGCCCGCGGCCTA-3', reverse: 3'-CGGTGTGTACAAGGCCCGG-5') (Hoorzook and Barnard, 2022). The PCR reaction was prepared in a final volume of 50 µL containing 2.5 units of Amplitaq DNA polymerase (Perkin Elmer), 200 µM of each dNTP, 1X PCR buffer, 25 pmol of each primer, and 3.0 mMMgCl$_2$ (Bharagava et al., 2009). Amplification was performed using an Applied BiosystemsVeriti® 96-well Thermal Cycler under 35 cycles with the following conditions: initial denaturation at 95°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 90 seconds, and final extension at 72°C for 7 minutes. The amplified products were purified with a gel extraction kit (Merk Biosciences, Bangalore) and sequenced at Chromous Biotech Pvt. Ltd. on an ABI 3500 Genetic Analyzer using Big Dye Terminator v3.1. The resulting sequences were analysed using the BLAST tool at NCBI (Gautam, 2022;Bruno et al., 2000), which confirmed the bacterial identity. A phylogenetic tree was generated using the Neighbor-joining method, and the sequences were deposited in Gene Bank under accession number PP830646.

2.5. Analysis of isolated bacterium growth and Cr^{6+} potential for reduction

The bacterium was cultivated in 250 mL Erlenmeyer flasks containing 100 mL of autoclaved LB broth supplemented with varying Cr^{6+} concentration (250 mg/L-2000 mg/L). The flasks were incubated at 35 ±2 °C with shaking at 120 rpm (Innova 4230, USA) to evaluate bacterial growth and Cr^{6+} reduction. LB broth with bacterial culture served as the positive control, while LB broth containing Cr^{6+} without bacteriawas used as the experimental control. Cr^{6+} reduction was analysedby comparing results with the experimental control. Additionally, a 24-hour bacterial culturewas prepared to obtain a6 × 10^2 CFU/mLinoculum. Bacterial growth was monitored using a spectrophotometer (Evolution 201, Australia)at 600 nm.The Cr^{6+} reduction potential was assessed calorimetricallyby measuring the decrease in Cr^{6+} concentration using the 1,5-diphenylcarbazide (DPC) method at 560 nm.The percentage of Cr^{6+} reduction was determined using the following formula (Mishra et al., 2021).

[Where, Ci = initial Cr^{6+} conc. (mg/L) and Cf= final Cr^{6+} conc. (mg/L)]

2.6. Scanning Electron Microscopic analysis (SEM)

The effects of Cr^{6+} on the morphology of the isolated bacterium were observed using Scanning Electron Microscopy (SEM). The bacteria were cultured in LB broth with 2000 mg/L Cr^{6+} for 24 hours using a 4×10^4 CFU/mL inoculum at 35 ±2 °C and 120 rpm in a shaking flask. Cells grown in LB broth without Cr^{6+} served as the control. After incubation, bacterial cells were collected by centrifugation at 8000 rpm for 10 minutes at 4°C. The "pellets were washed three times with phosphate-buffered saline (PBS, pH 7.0) and pre-fixed in 2.5% glutaraldehyde at 4°C for two hours. Following pre-fixation, the cells were washed twice with PBS (pH 7.2), post-fixation with 1% osmium tetroxide for one hour, and washed three times with PBS". The cells were dehydrated through a series of acetone concentrations: 20%, 40%, 60%, 80%, and 100% (v/v). The fixed cells were dried using a critical point dryer (CPD), coated with platinum via an ion sputter coater (JEOL, Japan JFC 1600 Auto Fine Coater) and examined under SEM (JEOL JSM-6490LV)to observe morphological changes (Tan et al., 2020).

2.7. Fourier transform infrared spectrophotometric (FT-IR) analysis

The LIWW sample was oven dried at 105 °C to analyse the functional group of organic compounds. The resulting pellets were mixed with Potassium Bromide (KBr) to obtain the absorption spectra. A 15-ton PCI Hydraulic Press (Manual) was used to combine the dried sample with KBr (IR Grade; purity ≥ 99%) in a 1:30 ratio. The mixture was thoroughly ground and pressed into a thin pellet (13 mm diameter, 1mm thickness). Absorbance spectra were recorded using a Nicolet FT-IR Spectrometer (Model Nicolet 6700, Thermo Fisher Scientific, USA) with 4 cm^{-1} resolution over 4000 to 400 cm^{-1}. Spectra were obtained by scanning in ambient air against a background spectrum of pure KBr. Data processing and analysis were performed using OMNIC™ software (v7.4), enabling identification of characteristic functional groups in the wastewater sample (Saran et al., 2025a).

2.8. Gas chromatography–mass spectrometry (GC-MS) analysis

2.8.1. Solvent-based extraction of organic pollutants from untreated and treated LIWW

Considering that LIWW effluent contained a complex mixture of compounds, a single solvent extraction method was insufficient to remove all contaminants. To effectively extract organic pollutants, GC-MS analysis was performed using a multiple solvent combination. Different organic solvent systems were applied in a liquid-liquid extraction (LLE) process to separate the pollutants from the effluent. Initially, 100 mL of centrifuged effluent was acidified to pH 2.0 using 1N HCl (centrifuged at 8000 rpm for 20 minutes at 4°C).The extraction was repeated three times with equal volume of two solvent systems: 50 mL of dichloromethane (DCM) mixed with 50 mL of diethyl ether (solvent1) and 50 mL of DCM mixed with 50 mL of n-hexane (solvent 2). Extractions were performed in a 500 mL separating funnel with shaken occasionally (Saxena et al., 2017). The organic solvent layer containing residual impurities were separated and vacuum evaporated at 40°C until completely dry. Prior to GC-MS analysis, the dried extract was re-dissolved in 2 mL DCM and filtered through a 0.22 µm syringe filter. All solvent were used were HPLC grade with a purityof ≥ 99%.

2.8.2. GC-MS/MS analysis

For trimethylsilyl derivatisation, the extracts were heated with BSTFA (N, O-bis (trimethylsilyl) trifluoroacetamide) containing 1% TMCS and heated at60°C for 15 minutes (Marco et al., 2007).GC-MSanalysiswas performed using a Turbo mass spectrometric detector coupled with a PE Auto System XL gas chromatograph(Thermo Fisher Scientific, Waltham, USA) (Chowdhary et al., 2022). The column temperature was initially set at 70°C (held for 4 minutes), then increased at a rate of 20°C per minute until it reached 305°C, where it was maintained for 25 minutes. Helium (He) was used as the carrier gas at a constant flow rate of 1 mL/min. Electron ionisation (EI) mass spectra were recorded at 70 eV in full-scan mode over a mass-to-charge ratio (m/z)of 50-800. Identification of compounds was achieved by comparing the acquired spectra to the National Institute of Standards and Technology (NIST, USA), library integrated within the system.

2.9. Statistical analysis

To increase analytical precision, each observation was performed in triplicate ($n = 3$), and the data were recorded. Statistical analysis was performed using IBM SPSS Statistics version 20. One-way Analysis of Variance (ANOVA) was applied, and the means were compared using Tukey's post hoc test ($p < 0.05$) to assess the significance of the results and the variability of the collected data.

3. Results and discussions

3.1. Physico-chemical characteristics of leather industry wastewater

Physico-chemical analysis of control (UTLIWW) and treated (*Micrococcus endophyticus* treated LIWW) samples showed significant changes in various parameters as presented in Table 1.Pollutants were substantially reduced, including alkalinity (94.34%), BOD (76.22%), COD (62.22%), TDS (47.13%), chloride (52.89%), calcium (60.36%), and magnesium (79.46%). Total Cr (2000 mg/L) analysis using AAS revealed a removal efficiency of 96.78%, resulting in a final concentration of 64.4 mg/L. These findings demonstrate the efficacy of *Micrococcus endophyticus* in evidently reducing both organic and inorganic contaminants in LIWW.

Previous studies demonstrated the effectiveness of bacterial strains in treating industrial wastewater. Maurya et al. (2025) reported that a consortium of *Bacillus vallismortis, Bacillus haynesii, Aeromonasaquatilis,* and *Enterococcus faecium* achieved substantial reductions in key pollutants, including COD (82.5%), BOD (92.7%), alkalinity (60.1%), and TDS (72.3%) in tannery wastewater. Similarly, Fajri et al. (2025) highlighted the efficiency of *Bacillus cereus* in textile wastewater treatment, achieving a COD reduction (80.1%) and colour removal (48.8%), while *Rossellomorea marisflavi* effectively reduced COD by 78.9%.

These studies indicated that bacterial strains played a pivotal role in enhancing wastewater treatment efficiency, particularly for industrial effluents from textile and LIs.

3.2. Isolated chromium resistant bacterium and its characteristics

Five morphologically distinct bacterial strains (AAKC1-AAKC5) were isolated from LIWW. Screening test revealed that only three strains AAKC1, AAKC3, and AAKC5 could tolerate of Cr^{6+} concentration up to 2000 mg/L. The remainingstrainsAAKC2 and AAKC4, failed to reduce even 250 mg/L of Cr^{6+} and were excluded from further studies. The unique morphological and biochemical characteristics of the selected isolated are summarized in Tables 2 and 3.

Molecular identification of the AAKC5 strain, based on 16S rRNA gene sequencing and BLAST analysis, confirmed it as *Micrococcus endophyticus* with accession number PP830646. The sequences analysis revealed 100% similarity to known *Micrococcus endophyticus* sequences, as illustrated in Fig. 1 (a) and (b), establishing AAKC5 as a potential Cr tolerant bacterium for subsequent bioremediation experiments.

Table 1: Physico-chemical parameters of untreated and treated leather industry wastewater

Parameters	Untreated LIWW	Treated by AAKC5	Permissible limit (CPCB,2013)
Colour	Dark-Brown	Light Brown	Colourless
Odour	Pungent	Pungent	No odour
pH	6.39±0.2	6.91±0.3	5.5–9.0
Temperature (°C)	28.5±0.3	30.2±0.4	40 °C
Conductivity (mS/cm)	20960±12.1	26534±14.2	-
Alkalinity (mg/L)	1925.29±18.6	109±9.3	500
BOD (mg/L)	1686±17.9	401±10.6	30
COD (mg/L)	5583±43.2	2109±32.6	250
TDS (mg/L)	3297±43.8	1743±28.9	2100
Chloride (mg/L)	130.13±12.9	61.3±9.7	1000
Calcium (mg/L)	108.72±9.2	43.1±6.3	-
Magnesium (mg/L)	550.18±23.7	113±8.5	-

[**Note:** All the reduction values are in relative percentages to their respective controls, also all the experimental values are mean value (n=3)]

Table 2 Morphological characteristics of isolated strains

Strains	Form	Surface	Colour	Margin	Elevation	Opacity
AAKC1	Circular	Smooth	Off white	Lobate	Convex	Opaque
AAKC3	Curled	Smooth	White	Entire	Flat	Translucent
AAKC5	Curled	Smooth	Yellow	Lobate	Raised	Opaque

Table 3 Biochemical characteristics of isolated strains

Strains	Gram staining		Catalase	Motility	H_2S	Indole	Citrate	Urease	MR	VP	Starch
	Staining	Shape									
AAKC1	-	Bacilli	+	-	-	-	+	+	+	-	+
AAKC3	+	Bacilli	+	-	-	-	+	+	+	-	+
AAKC5	+	Cocci	+	-	-	-	-	+	+	-	-

[**Note:** present (+), and absent (-) for that particular test]

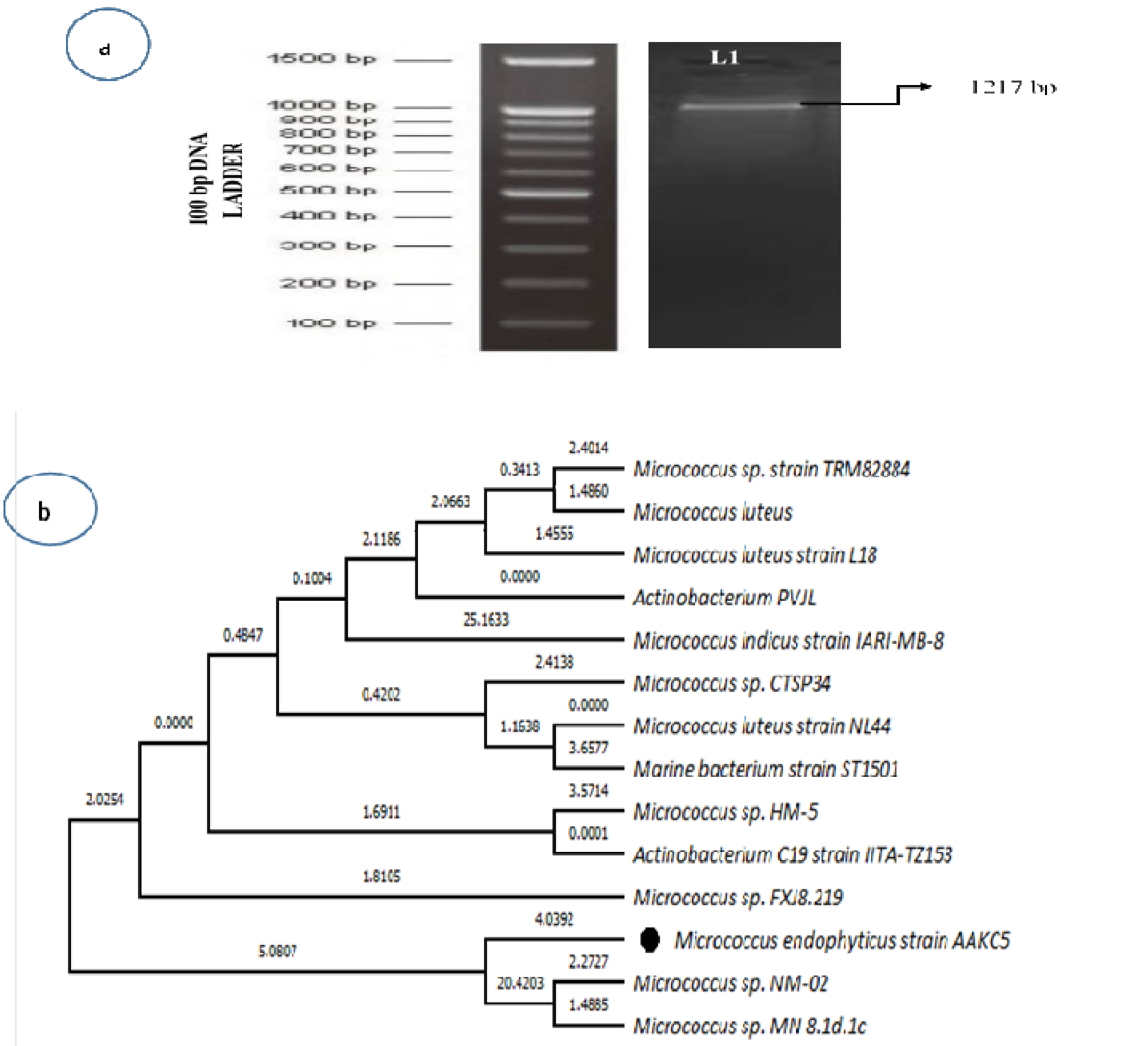

Fig. 1(a): Agarose gel analysis of DNA (PCR product) of Cr resistant bacteria. Lane 1: AAKC5 with marker DNA (100 bp DNA ladder);b: Phylogenetic tree showing the relationship of isolated bacterium AAKC5 with their neighbouring sp.

3.3 Growth pattern and chromium reduction by isolated bacteria

In the current study, bacterial growthwas influenced by Cr^{6+} concentrations ranging from 250 to 2000 mg/L. At 250-500 mg/L, all three bacterial strains (AAKC1, AAKC3, and AAKC5) exhibited growth after 96 hours, as shown in Fig. 2.

When the concentration wasincreased to1000, 1500, and 2000 mg/L, strainsAAKC1 and AAKC3exhibited growth after 72 hours, while strain AAKC5was found to grow after 84 hours. This indicated that bacterial growth was inhibited at higher Cr^{6+} concentrations. Furthermore, *Micrococcus endophyticus* (AAKC5) showed a concentration-dependent Cr^{6+} reduction pattern, as shown in Fig. 3, the reduction efficiency declined with increasing Cr^{6+} concentration.

In this study, the reduction potential of the isolated bacterium was assessed across a gradient of Cr^{6+}concentrations (250–2000 mg/L), as shown in Fig. 3. The bacterium was found to reduce Cr^{6+} by 89.9% (250 mg/L), 86% (500 mg/L), 84.03% (1000 mg/L), 81.49% (1500 mg/L), and 81.02% (2000 mg/L) over 96-120 hours, respectively. The observed inverse relationship between bacterial growth and higher Cr^{6+} concentration was attributed to the toxic effects of Cr^{6+}. After a period (120 hours), the bacterial growth curve reached saturation, suggesting that the bacterial isolate developed adaptive mechanisms to survive and resist Cr^{6+}toxicity. Elevated Cr^{6+} concentrations generate oxidative stress and DNA damage, impairing bacterial metabolism and viability. This likely explains the reduced Cr^{6+} reduction efficiency at higher concentration (Chen and Tian, 2021). There results highlight the ability of the strain to tolerate and partially detoxify Cr^{6+} in wastewater.

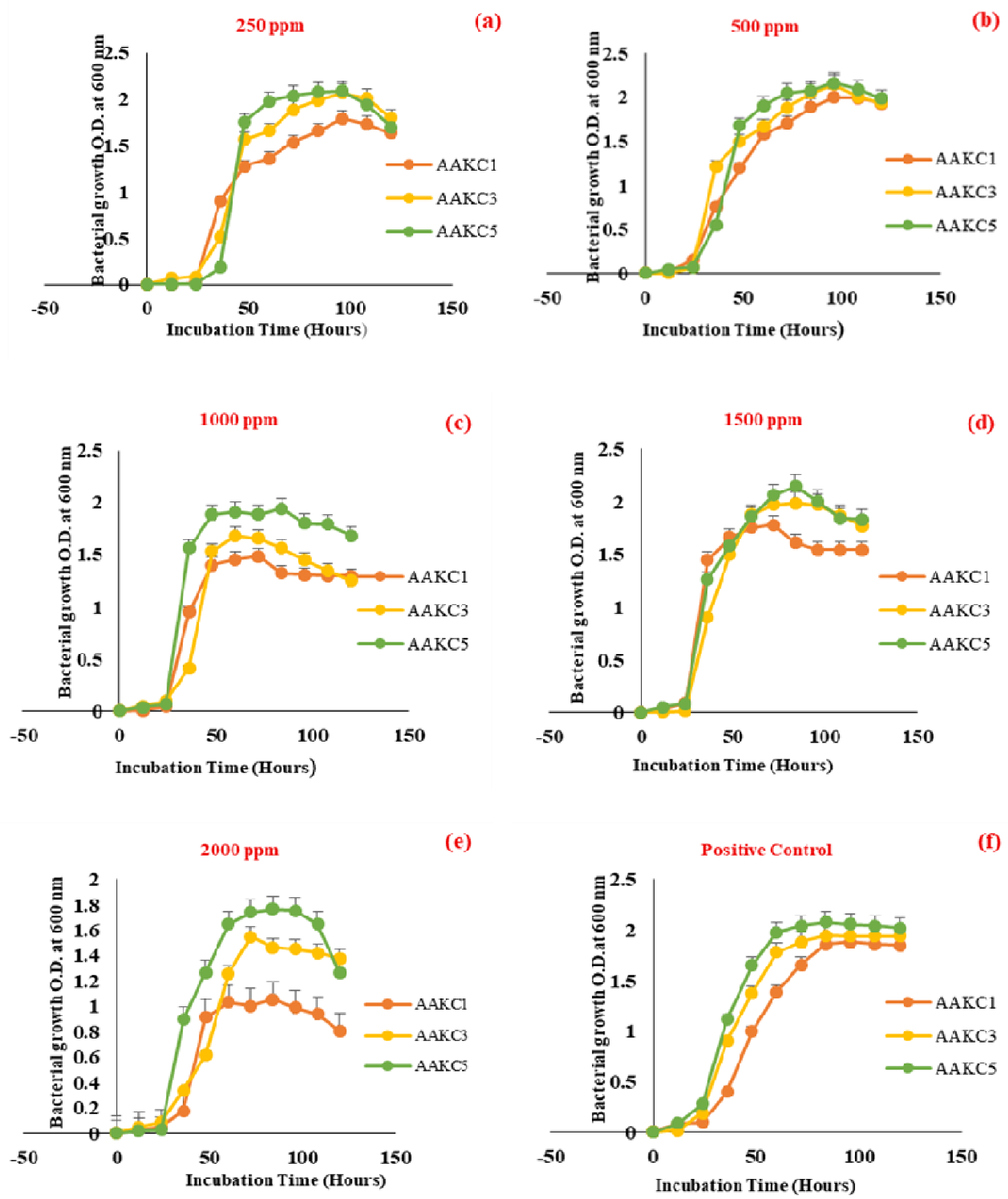

Fig. 2Growth curves pattern of isolated strains (AAKC1, AAKC3 and AAKC5) at 250 mg/L, 2000 mg/L (from a-e) Cr^{6+} concentration with positive control (f).

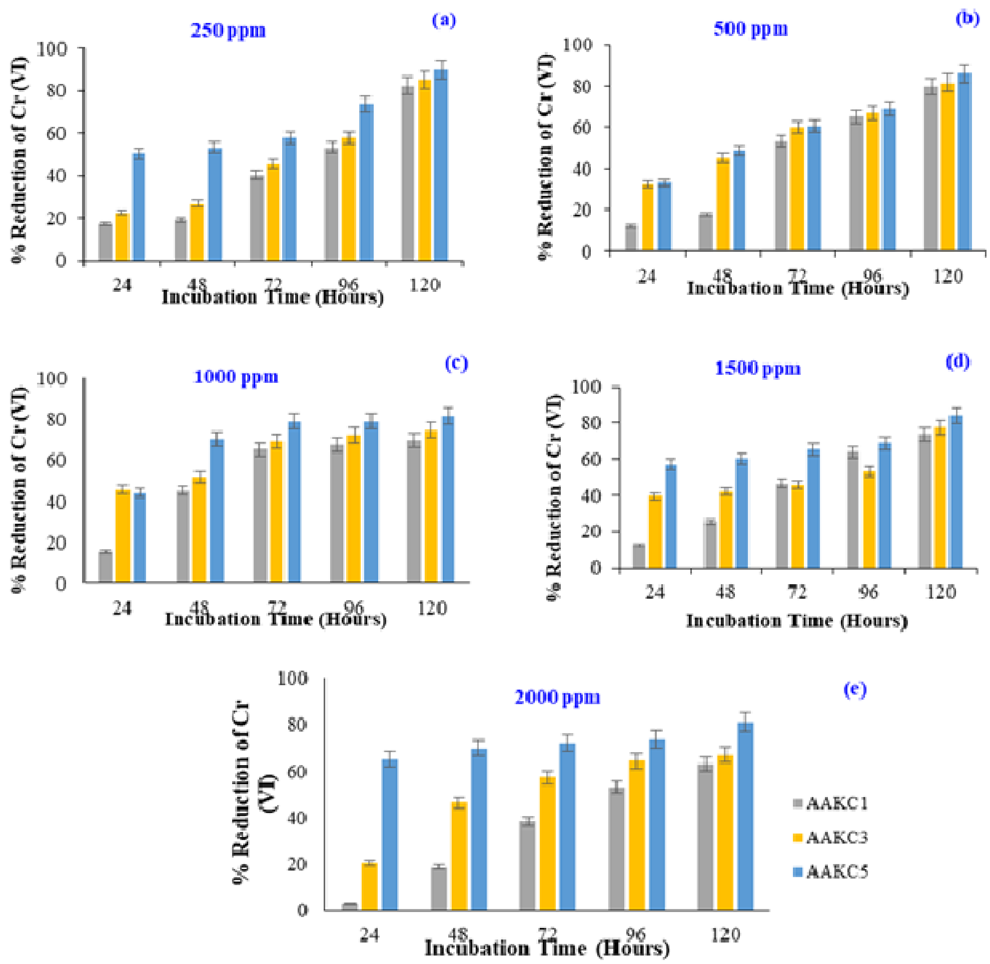

Fig. 3Chromium reduction (%) of isolated strains (AAKC1, AAKC3 and AAKC5) at 250 mg/L to 2000 mg/L Cr^{6+} concentration.The difference between treatments is statistically different and significant determined by using one-way ANOVA. In each graph (a-e) above the bars represents the significantly differences according to ANOVA followed by the (post-hoc) Tukey's test (p<0.05)

*Micrococcus endophyticus*was found to reduce Cr^{6+} at concentrations up to 2000 mg/L. Previous studies have reported similar Cr reduction capacities in related bacterial species. Khanam et al. (2024) demonstrated that certain *Micrococcussp.* reduced $Cr^{6+}$by 99.81% (50 mg/L), 99.48% (100 mg/L), 91.30% (200 mg/L), 88.22% (300 mg/L), 27.69% (500 mg/L), and 11.35% (1000 mg/L). Mishra et al. (2021) observed that *Microbacterium paraoxydans* reduce $Cr^{6+}$by 39.24% (500 mg/L) and 94.45% (100 mg/L). Bharagava and Mishra (2018) reported the *Cellulosimicrobium sp.* reduced Cr^{6+} by 99.33% and 96.98% at 50 and 100 mg/L at 24 and 96 hours, respectively. While only 84.62% and 62.28% reductions occurred at 200 and 300 mg/Lafter 96 hours. Similarly, Zahoor and Rehman (2009) found that *Bacillus sp.* JDM-2-1 reducedCr^{6+} by 40% at 24 hours, 66% at 48 hours, 77% at 72 hours and 85% at 96 hours, whereas *S. capitis* achieved 29%, 53%, 65%, and 81% reductions at the same respective time intervals. These studies collectively highlight the significant but concentration and time dependent Cr reduction potential of various bacterial strains.

Bacteria were believed to reduceCr^{6+} through the acid adsorption mechanism, which requires sufficient protons (H^+) in the medium to initiate anions exchange, facilitating Cr^{6+}uptake and reduction. Upon accumulations, Cr^{6+}acted as a terminal electron (e^-) acceptor, reducing to Cr^{3+} and binding to the bacterial cell wall (Rahman and Thomas, 2021). Two mechanisms, namely biosorption on the microbial cell surface and bioaccumulation within cells, were responsible for the Cr reduction. Bioaccumulation occurred in two stages: an initial rapid phase involving physical adsorption or ion exchange on the cell surface, followed by a slower phase where $Cr^{6+}$was transported intracellularly through active metabolism (Devi et al., 2025b). Biosorption processes were metabolism-dependent and involved ion exchange, adsorption, chelation, and complexation mechanisms, enabling effective removal of heavy metals from the environment (Priya et al., 2022; Bharagava and Mishra, 2018).

Due to the chemical resemblance between the two oxyanions, chromate (CrO_4^{2-}) ions were reported to cross biological membranes through the sulphate transport pathway (Qasem et al., 2021). Inside bacterial cells,$Cr^{6+}$was rapidly converted to Cr^{3+} through enzymatic or non-enzymatic processes, which could exert toxic effects within the cytoplasm. Bacteria reduced Cr^{6+} to Cr^{3+} under both aerobic and anaerobic conditions using an electron transport system (ETS) involving cytochromes. In aerobic conditions, short-lived intermediates such as Cr^{5+} and Cr^{4+} were produced during the two to three-step reduction to the stable end product Cr^{3+}form (Saran et al., 2023); e^- and NADPH from endogenous reserves acted as electron donors for Cr^{6+} reduction. However, it remains unclear whether the conversion from Cr^{5+} to Cr^{4+} and Cr^{4+} to Cr^{3+} occurred spontaneously or was mediated by specific enzymes (Ramli et al., 2023).

3.4. SEM-EDX analysis

After 96 hours of bacterial treatment with and without 2000 mg/L of $K_2Cr_2O_7$, the cell morphology of *Micrococcus endophyticus* (AAKC5) was observed with a Scanning Electron Microscopy (SEM). As shown in Fig. 4(a) for the control and Fig. 4(b) at 2000 mg/L of $K_2Cr_2O_7$, the observed morphological changes were likely due to the adsorption or precipitation of reduced Cr^{3+} on the bacterial cell surface. The AAKC5 cells at 2000 mg/L exhibited adiameter of 949.46 nm with slight projections emerging from their spherical shape, whereas cells without $K_2Cr_2O_7$ maintained a smooth cocci surface with a diameter of 931.85 nm.

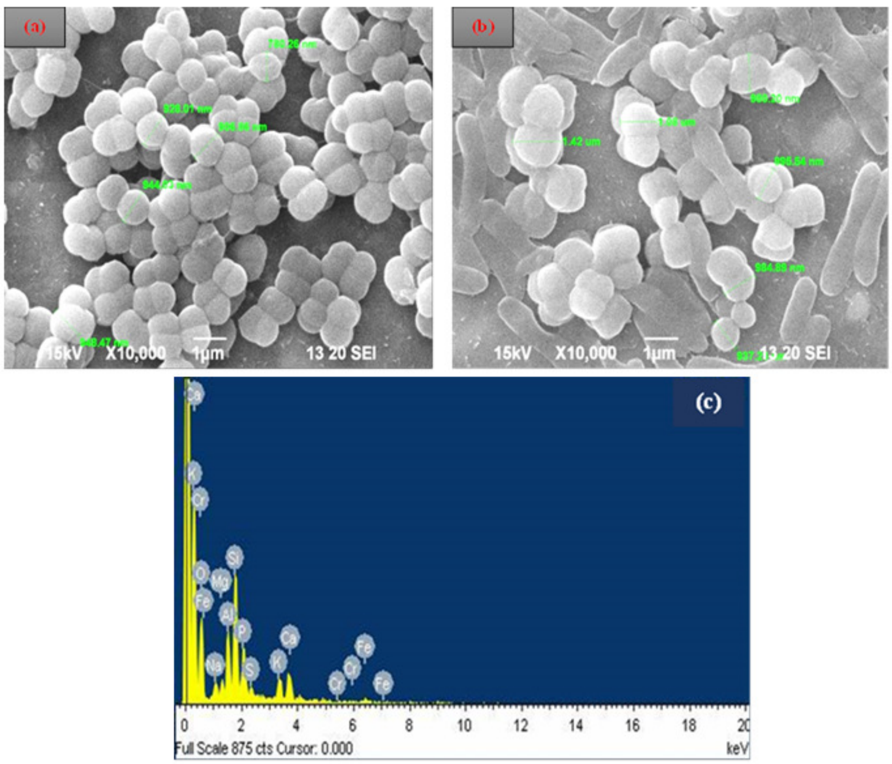

Fig.4: SEM analysis (a) control, (b) *Micrococcus endophyticus* (AAKC5) and (c) EDX analysis of *Micrococcus endophyticus* (AAKC5)cells grown in the presence of 2000 mg/L of $K_2Cr_2O_7$

SEM analysis suggested that Cr reduction by *Micrococcus endophyticus* primarily involved the transformation $Cr^{6+}$to Cr^{3+}through multiple enzyme-mediated mechanisms .Reductases such as ChrR and YieF functioned as e^- donors,utilizing endogenous e^- and NAD(P) H during both aerobic and anaerobic phases. In the anaerobic phase, Cr^{6+} reduction was facilitated by membrane-associated and soluble enzymes, while certain anaerobes metabolites, including H_2S, acted as effective Cr^{6+}reductants, highlighting the strains versatile Cr biotransformation capabilities (Khan et al., 2025).

Energy Dispersive X-ray (EDX) analysis revealed Cr peaks in AAKC5 cells exposed toCr^{6+}, with a content of 0.015 wt. %, as shown in Fig. 4(c). These peaks were attributed to the precipitation of reduced Cr^{3+} on the cell surface or complexation of Cr^{3+} with cellular molecules. As shown in Table 4, elements such as Oxygen (O), Sodium (Na), Magnesium (Mg), Phosphorus (P),Calcium (Ca), Chromium (Cr), and Potassium (K) contributed to Crsurfacelocalisationon bacterial cells (Suresh et al., 2021). These finding highlighted the adaptive response of bacteria to Cr^{6+} stress and confirmed their potential for bioremediation through Cr reduction and immobilisation, supporting sustainable wastewater treatment applications.

Table 4 Elemental content in bacterial isolate AAKC5 grown on LB agar amended with 2000 mg/L Cr^{6+}

Element	Weight%	Atomic%
O K	63.091	76.98
Na K	4.01	3.41
Mg K	2.45	1.97
P K	18.91	11.91
K K	10.01	5.00
Ca K	1.49	0.73
Cr K	0.039	0.015
Total	100	

3.5. FT-IR analysis

Multiple peaks at different wave numbers were observed in the FTIR spectra of the control (UTLIWW+ 2000 mg/L Cr^{6+} concentration) and treated (*Micrococcus endophyticus* treated LIWW at 2000 mg/L Cr^{6+} concentration) samples, as shown in Fig. 5 (a) and (b).FTIR analysis revealed the presence of several functional groups, including alcohol (O-H), alkene (C=C), aldehyde (C-H), alkynes (C≡C), fluro-compound (C-F), amine (C-N), amines ($-NH_2$), and halo compounds (C-Br and C-Cl) with infrared wave numbers ranging from 4000 to 500 cm^{-1}. Peaks observed at 3420.8 and 3430.5 cm^{-1} were attributed to O-H stretching of alcohol, suggesting overlapping amine and hydroxyl stretching on the bacterial cell surface (Saran et al., 2025a).The asymmetric protein stretching of nucleic acid, polysaccharides, and lipids in the bacterial cell wall was slightly altered, as indicated by absorption peaks at 2888.5 and 28885.8 cm^{-1}in both the control and treated groups. Peak observation at 2824.4 and 2827.2 cm^{-1} corresponded to aldehyde C–H stretching.

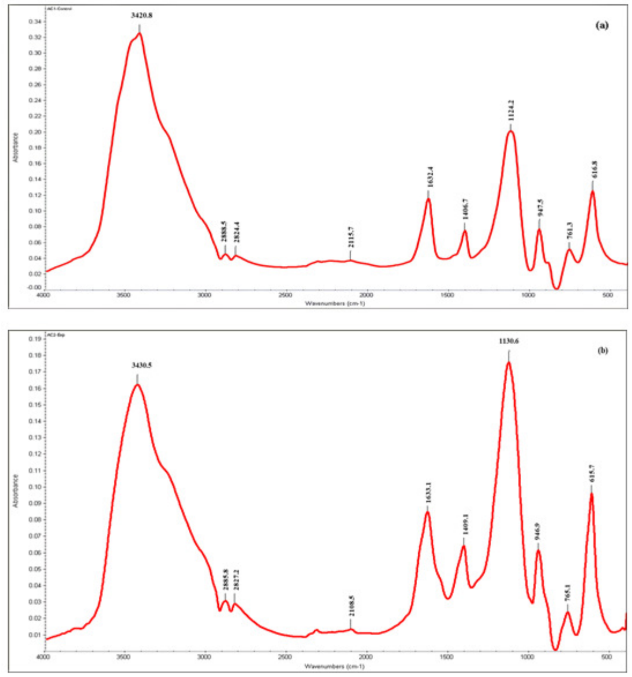

Fig.5 FT-IR analysis (a) untreated LIWW+ 2000 mg/L Cr^{6+} and (b) *Micrococcus endophyticus* (AAKC5) treated LIWW at 2000 mg/L Cr^{6+}

Additionalprominent absorption peaks appeared at 1632.4-1633 cm^{-1} (C=C stretching, alkene) (Devi et al., 2025a) and 1406.7-1409.1 cm^{-1}(O–H bending, alcohol), 2115.7-2108.5 cm^{-1} (C≡C stretching, alkyne) cm^{-1}, 1124.2cm^{-1}(C–N stretching, amine), and 1130.6 cm^{-1}(C–F stretching, fluoro compound). The peak at 761.3-765.1 cm^{-1} (indicating -C-H stretching) becomes more distinct in Cr^{6+}exposedbiomass, indicating a shift from 616.8-615.7 cm^{-1}(Saran et al., 2025a) and suggesting involvement of -SO_3H groups in Cr-sulphonic interaction (Bharagava and Mishra, 2018), as summarized in Table 5.

Table 5 Fourier-transform infrared (FTIR) spectroscopy analyses

Wavenumber (cm^{-1})	Functional group	Class	Peak details	Control	Treated
3430.5	O-H stretching	Alcohol	Strong, broad	-	+
3420.8	O-H stretching	Alcohol	Strong, broad	+	-
2888.5	N-H stretching	Amine salt	Medium	+	-
2885.8	N-H stretching	Amine salt	Medium	+	-
2827.2	C-H stretching	Aldehyde	Medium	-	+
2824.4	C-H stretching	Aldehyde	Medium	+	-
2115.7	C≡C stretching	Alkyne	Weak	+	-
2108.5	C≡C stretching	Alkyne	Weak	-	+
1633.1	C=C stretching	Alkene	Medium	-	+
1632.4	C=C stretching	Alkene	Medium	+	-
1409.1	O-H bending	Alcohol	Medium	-	+
1406.7	O-H bending	Alcohol	Medium	-	+
1130.6	C-F stretching	Fluoro compound	Strong	-	+
1124.2	C-N stretching	Amine	Strong	+	-
947.5	C=C bending	Alkene	Medium	+	-
946.9	C=C bending	Alkene	Medium	-	+
765.1	C-H bending	Monosubstituted	Medium	-	+
761.3	C-H bending	Monosubstituted	Medium	+	-
616.8	C-Br stretching	Halo compound	Medium	+	-

615.7	C-Cl stretching	Halo compound	strong	-	+

[**Control:** Untreated leather industry wastewater at 2000 mg/L Cr^{6+}; **Treated:***Micrococcusendophyticus* treated leather industry wastewater at 2000 mg/L Cr^{6+}; **+:** Present; **-:** Absent].

3.6. Changes in organic and inorganic composition in untreated and treated LIWW by GC-MS analysis

The study focused on detecting significant patterns and differences in the chemical composition of the control (UTLIWW + 2000 mg/L Cr^{6+} concentration) and treated samples (*Micrococcus endophyticus* treated LIWW at 2000 mg/L Cr^{6+} concentration). Compounds were measured based on their retention time (RT) during the analysis. The control sample exhibited 11 organic compounds peaks,whereas the treated sample showed 17 peaks. The chemical compound identified by GC-MS are summarized in Table 6, and the corresponding chromatogram are presented in Fig. 6 (a and b). The Retention Time (RT) indicated the elution time of each compound during the GC-MS analysis. GC-MS analysis of the control and treated samples revealed significant variations in the chemical composition. The control sample showed the predominance of hazardous compounds associated with leather finishing and dyeing processes, including 2-[2-(2-methoxyethoxy) ethoxy]-ethanol (RT 6.25), oxiranemethanol, 2-phenyl (RT 6.47), and benzene derivatives (RT 6.66-6.75)(Saran et al., 2025a). Furthermore, higher concentrations of synthetic tanning chemicals (RT 43.19, 52.36) and phthalic acid esters (RT 30.99-31.97) indicated the persistence of pollutionin the untreated effluent (Bharagava et al., 2018).The treated sample, in contrast, showed a significant reduction in hazardous compounds and an increase in biodegradable fatty acids, including tetradecanoic acid (RT 42.65), eicosanoic acid (RT 42.25), and hexadecanoic acid (RT 34.36). The significant decreases of toxic substances such as phosphorothioic acid (RT 28.35) and cyclotetrasiloxane (RT 12.90) demonstratedthe effectiveness of *Micrococcus endophyticus* (AAKC5) in degrading hazardous pollutants. The predominance of naturally occurring fatty acids over synthetic tanning agents further supported the potential of microbial bioremediation to improve the quality of wastewater. The effectiveness of *Micrococcus endophyticus* (AAKC5) in detoxifying LIWW with 2000 mg/L Cr^{6+} was evaluated using GC-MS analysis. The treated sample showed a significant reduction in harmful organic compounds, detected in the untreated wastewater included benzene derivatives, phthalic acid esters, glycol ethers, epoxide-containing compounds, and siloxanes, demonstrating the potential of microbial bioremediation for sustainable wastewater management in the LIs.

4. Conclusion

This study established the isolation of Cr resistant bacterium from LIWW heavily contaminated with $Cr^{6+.}$ The identified strain, Micrococcus endophyticus (AAKC5), demonstrated significant bioremediation potential by achieving 81.02% reduction of Cr^{6+} (2000 ppm) within 96-120 h at 35 ± 2 °C, with maximum activity occurring during the exponential growth phase. SEM analysis confirmed surface-associated Cr accumulation, while FT-IR spectra highlighted the involvement of amino and carboxyl functional groups in Cr6+ detoxification. GC-MS revealed notable metabolic alterations associated with the reduction process. These findings demonstrated that the isolated bacterium efficiently reduced or detoxified Cr6+ in effluent environments with high Cr concentration and harsh conditions. Strain AAKC5 achieved 81.02% reduction of Cr6+ at an initial concentration of 2000 ppm at 35±2 °C, even with low biomass, highlighting its suitability for cost effective industrial wastewater treatment and strong Cr resistance. Therefore, it could serve as a potential candidate for efficient bioremediation of metal-contaminants sites, contributing to environment protection and humans and animal health.

Acknowledgement

The authors sincerely thank the Department of Environmental Microbiology and USIC, Babasaheb Bhimrao Ambedkar University, Lucknow, for providing the necessary facilities and support to carry out this research.

Author contribution

Christina Saran: Conceptualization, Drafting, Writing and Revision of article, Validation, Data Curation, Editing, Revision, Visualization and final approval of the version to be submitted.
Arya Arun Kumar: Writing and Revision of article, Data Curation.
Anuradha Devi: Editing and Language improvement and final approval of the version to be submitted,
Luiz Fernando Romanholo Ferreira: DataCuration, Editing, Revision and Visualization, **Ganesh DattatrayaSaratale:** Editing and Language improvement, **Ram Naresh Bharagava:** Supervision, Conceptualization, Draft preparation, Editing, Revision, Review, Finalization & final approval of the version to be submitted, Resource management.

Conflict of interest

The authors declare that there are no conflicts of interest**.**

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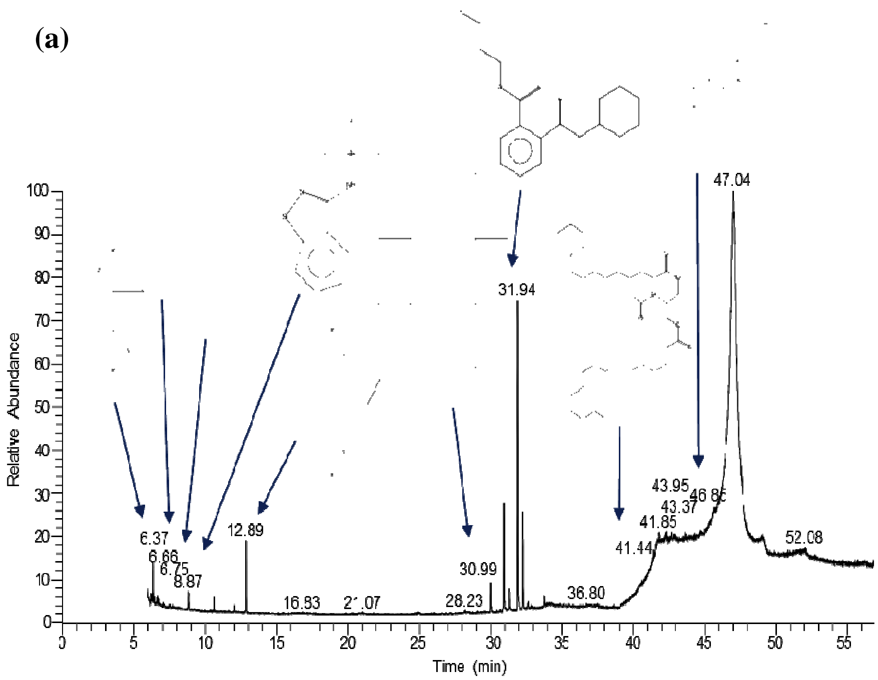
Table 6 GC-MS analyses untreated and treated wastewater sample collected from leather industry

RT	Compound Name	Control	Treated	Source	Toxicity
6.25	2-[2-(2-methoxyethoxy) ethoxy]-ethano	-	+	Leather finishing	Low acute toxicity
6.37	3-Hydroxyisovaleric acid	+	-	Liming &unhearing processes	Eutrophication
6.47	Oxiranemethanol, 2-phenyl	-	+	Leather finishing&dyeing	Skin & respiratory irritation
6.66	Benzene, 1-ethyl-3-methyl	+	-	Dyes & degreasing agents	Volatile organic compounds
6.68	Benzene, 1,2,3-trimethyl	-	+	Leather finishing & dyeing	Skin & respiratory irritation
6.75	Benzene, 1-ethyl-4-methyl	+	-	Found in degreasing agents	Bioaccumulation potential
8.87	Hexanoic acid	+	-	Soften leather	Elevates BOD & COD
10.64	1,2-Benzisothiazol-3-amine	-	+	Preservative chemicals for leather	Persistent in the environment
12.89	2,6-Dihydroxyacetophenone	+	-	Syntans& dye intermediates	Bioaccumulation potential
12.90	Cyclotetrasiloxane, Octamethyl	-	+	Leather finishing formulations	Volatile organic compound
28.35	Phosphorothioic acid	-	+	Leather preservative	Potential human toxicity
30.99	Phthalic acid, butyl hex-3-yl ester	+	-	Leather finishing coatings	Persistent pollutants
31.94	1,2-Benzenedicarboxylic acid, butyl octyl ester	+	-	Plasticizer in coatings	Toxic to aquatic life
31.97	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	-	+	Flexible leather coatings	Persistent pollutant
32.33	Isobutyl 2,4,4-trimethylpentyl ester	-	+	Coatings & leather finishing	Endocrine disruptor
34.36	Hexadecanoic Acid	-	+	Fat liquoring agents	Oxygen depletion
40.9	4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester	-	+	Tanning agents& dyes	Bioaccumulate properties
41.44	Glycerol 2-acetate 1,3-dipalmitate	+	-	Degreasing in leather processing	Sludge formation
42.25	Eicosanoic Acid, 2-[(1-oxohexadecyl) oxy]-1-[[(1-oxohexadecyl) oxy] methyl] ethyl Ester	-	+	Provide softness& flexibility	Persistent in the environment
42.65	Tetradecanoic Acid	-	+	Finishing agents	Bioaccumulation
43.19	Phthalic acid	-	+	Synthetic tanning agents	Potential carcinogenic
43.37	4-(N,N-Dimethylaminomethyleneamino)-5,7-dinitrobenzofurazan	+	-	Dyes & colouring agents	Acute & chronic toxicity
43.95	Cyclodecasiloxane, eicosamethyl	+	-	Leather finishing processes	Volatility
44.03	1,2-Benzenedicarboxylic Acid, Dinonyl Ester	-	+	Tanning, & dyes agents	Endocrine disruptor
46.85	Cholestane-3,5-diol, 5-acetate, (3α,5β)	+	-	Processing of hides in tanneries	Slow degradation rates
47.63	Decanoic Acid	-	+	Softening agents	Bioaccumulation
52.36	2,5-Dihydroxyacetophenone	-	+	Synthetic tanning agents	Organic pollution
55.51	Hexadecanoic acid ester	-	+	Soften leather	Bioaccumulation

[**Note: Control:** Untreated leather industry wastewater at 2000 mg/L Cr^{6+}concentration;
Treated:*Micrococcusendophyticus* treated leather industry wastewater at 2000 mg/L Cr^{6+}; **(+):** Present; **(-):** Absent.

(a)



(b)

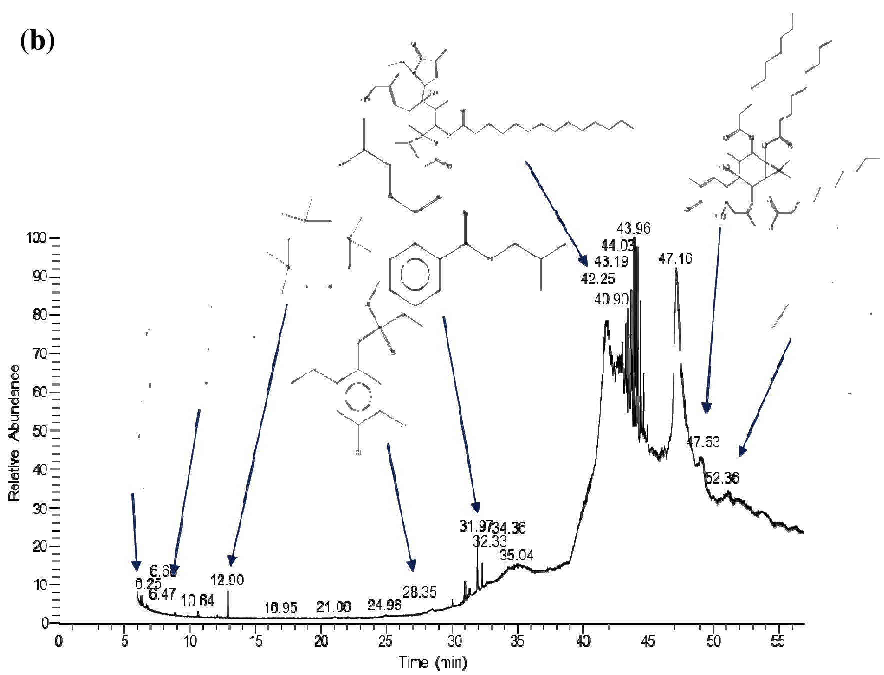


Fig. 6 GC–MS chromatogram (a) Control: UTLIWW sand (b) *Micrococcus endophyticus* (AAKC5) treated LIWW at 2000 mg/L Cr^{6+}

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Cite this article:

Saran, C., Kumar, A.A., Devi, A., Ferreira, L.F. R., Saratale, G.D., Bharagava., R.N., 2025. Reduction of hexavalent chromium by *Micrococcus endophyticus*isolatedfrom leather industry wastewater. J. Appl. Sci. Innov. Technol. 4 (2), 64-78.