Hexavalent chromium bioreduction by chromium-resistant sporulating bacteria isolated from tannery effluent

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ARTICLE INFO	ABSTRACT
	The main polluting source of heavy metal contamination of water is the leather tanning industry, which uses chrome powder and discharges it into the nearby ecosystem. In this investigation, chromium-resistant bacterial strains were isolated and characterized from tannery effluent. Based on morphological and biochemical characterization, the predominant sporulating *Bacillus sp.* was isolated and identified as *Bacillus subtilis* based on 16S rRNA gene sequencing. Chromium degradation by the bacterial strain was evaluated using the flask culture method at three different concentrations (300, 600, and 900 µg/ml) of Cr (VI), and the reduction potential of the isolated bacterium was analyzed by Atomic Absorption Spectrophotometry. A maximum reduction of approximately 78% was found at 24 hrs of incubation at pH 7 and at a constant temperature of 30°C. More than 50% of the Cr(VI) was decreased in 24 hours when the Cr(VI) concentration varied from 300 to 900 g/ml. FTIR analysis showed the involvement of hydroxyl and amine groups in chromium adsorption. As an outcome, this strain could be a promising bioagent for the environmentally friendly elimination of toxic Cr(VI) from polluted environments.

Introduction

Rapid urbanization has elevated the growth of industries at an exponential rate (Seragadam *et al.,* 2021 Bhutiani *et al.,* 2021a&b; Bojago *et al.,* 2023). There is a significant threat to the current environment because of the inappropriate discharge of industrial effluents with high concentrations of heavy metals into natural water resources and soil (Nagajyoti *et al.,* 2010; Bhutiani and Ahamad, 2018; Bhardwaj *et al.,* 2020; Ruhela *et al.,* 2022; Ahamad *et al.,* 2022;). A class of pollutants known as heavy metals accumulates in the food chain and in living things because they are not biodegradable (Kobya *et al.,* 2005; Ruhela *et al.,* 2019). This has jeopardized the environment's ability to support life. Toxic heavy metals such as chromium, cadmium, mercury, arsenic, and lead are major environmental pollutants due to their negative effects on both living ecosystems and public health (Ray and Ray, 2009; Bhutiani *et al.,* 2022). Cadmium, lead, mercury, nickel, copper, chromium, cobalt, and zinc are the heavy metals that the WHO (2010) study lists as being of the most concern (WHO, 2010). Chromium (Cr) and its derivatives cause many industrial processes to pollute the environment, including mining, chrome plating, pigment production, petroleum refining, leather tanning, wood preservation, textile production, pulp processing, and electroplating. (Wang & Xiao,1995)*.* The primary source of chromium contamination in the immediate environment is the tannery industry. Due to the wet process used in tanning hides and skin, which generates 30-35 L of effluent for every kg of treated skin or hides, tanning factories need an enormous amount of water (Nandy *et al.,* 1999). There are approximately 3000 tanneries in India, with the major tanning clusters in Tamil Nadu being Ambur, Vaniyambadi, Pallavaram, Pernambut, Ranipet, Dindigul, and Trichy (Kavitha and Ganapathy, 2015). Tannery effluent contains poisonous solid and liquid heavy metal waste that is discharged after treatment (Muhammad *et al.,* 2015).

Chromium (Cr) is a widespread contaminant that may be found in practically every aspect of the ecosystem, including the air, land, water, and biological systems (Rahman & Singh, 2019). Cr exists primarily in the ecosystem as trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. Due to the emergence of hydroxide and oxide compounds, Cr(III) is relatively insoluble at environmentally relevant pH (James *et al.,* 1997), and it stands for a crucial nutrient needed for healthy fat and sugar metabolism. (Cefalu & Hu, 2004). Cr (VI) has much higher solubility, mobility, bioavailability, and toxicity than Cr(III) (Cervantes *et al.,* 2001), is structurally similar to sulfate (SO_4^{2-}), is readily used up by bacterial and mammalian cells via the sulfate transport system and undergoes instantaneous mitigation reactions that result in the creation of various reactive intermediates (Cervantes *et al.,* 2001). Due to its great solubility in water and ability to be transported, Cr(VI) is 100 times more hazardous than Cr(III) (Katsayal *et al.,* 2022). Due to its rapid permeability and strong oxidizing nature, Cr(VI) is more harmful than Cr(III), which can cause severe damage to proteins and nucleic acids (Mishra *et al.,* 2012) and is extremely noxious to living organisms, causing allergies, eczema, irritations, and respiratory tract disorders. It has also been designated as a priority pollutant by the US Environmental Protection Agency (EPA,1998).

Over the past few years, conventional chemical or physicochemical treatment procedures such as adding lime, ion exchange, membrane separation, and adsorption followed by chemical precipitation and coagulation as $Cr(OH)_3$ have been described. However Heavy metals such as Cr(VI) removed from industrial effluents using conventional metal removal techniques have significant drawbacks,, such as high energy requirements, incomplete metal removal, and the accumulation of a large amount of toxic waste sludge (Ahalya *et al.,* 2003). Bioremediation has become a viable substitute for conventional physicochemical techniques. By using biological agents, either in situ or ex situ, bioremediation is used for eliminating inorganic contaminants from contaminated environments such as water, soils, sludge, and waste streams. (Sundari, 2017).

Many bacteria have been found to convert hexavalent chromium to trivalent chromium. (Wang *et al.,* 1989) (Dong *et al.,* 2013) (Shen & Wang 1993) (Bopp *et al.,* 1983) (Camargo *et al.,* 2003. In recent studies, several more microorganisms, such as strains of *Pseudomonas* (Wani *et al.,* 2019), *Cellulosi microbium* (Bhargava & Mishra 2018), *Bacillus* (Li *et al.,* 2020), *Staphylococcus* (Ahmad *et al.,* 2022), and *Stenotrophomonas* (Sundari, 2017), have been shown to have significant alleviative effects on chromium. Compared to traditional physical and chemical approaches, the use of Cr(VI)-resistant bacteria for the detoxification of environmentally harmful chromium has been deemed to be more cost-effective, efficient, and secure. (Ganguli & Tripathi 2002) (Cheung & Gu 2007)(Polti *et al.,* 2009)(Piñón-Castillo *et al.,* 2010). Many species exploit hazardous Cr(VI) reduction to nontoxic Cr (III) form as one of their survival strategies in Cr (VI) polluted effluents. Acinetobacter and *Ochrobactrum arthrobacter*, *Serratia marcescens*, *Bacillus spp.*, *Pseudomonas fluorescens* LB300, Intrasporangium sp. Q5-1, *Enterobacter cloacae*, *Bacillus sp.* ES29, and *E. coli* (Camargo *et al.,* 2003, Bopp *et al.,* 1983, Wang *et al.,* 1989, Sheng and Wang, 1993). Furthermore, a combination of microorganisms can lower the mobility and toxicity of Cr(VI) by converting it to a less mobile and less toxic form of Cr(III). Because of the involvement of carboxyl, hydroxyl, and amine functional groups in the complexation of chromium metals, microorganisms have a high metal binding capacity for chromium. (Chatterjee *et al.,* 2011)

The study's objective was to identify and characterize a Cr(VI)-resistant bacterial strain with the ability to reduce Cr(VI) in tannery effluent. They were efficiently tested for chromium (VI) bioreduction.

Material and Methods
Study area and collection of sample
The effluent sample from the Begumpur Leather processing industrial area of Dindigul district, Tamil Nadu (longitude 77.9695°E, latitude 10.3624°N), was collected in a labeled presterilized screw-capped bottle, placed in an icebox and immediately transferred to the laboratory. The sample was kept at 4°C, analyzed, and used within

6 hours of collection. The collected effluent was subjected to physicochemical parameter analysis, and the findings were tabulated.

Heavy metal evaluation in tannery wastewaters
The estimation of trace heavy metals such as cadmium, chromium, cobalt, copper, iron, lead, manganese, magnesium, nickel, and zinc in the industrial effluent was estimated by atomic absorption spectrophotometry (AAS) using the standard method.

Isolation of chromium-resistant microorganisms: To isolate chromium-resistant bacteria, the industrial wastewater sample was serially diluted up to 10-8 dilutions, and 100 μl of each dilution was inoculated into a Luria Bertani (LB) agar plate containing 50 μg/ml potassium dichromate ($K_2Cr_2O_7$) using the spread plate method (Zahoor & Rehman 2009) and incubated at 37°C for 24 hours. Colonies with morphological differences were chosen and subcultured on a Luria Bertani agar plate containing 100 μg/ml potassium dichromate ($K_2Cr_2O_7$) to isolate and enumerate the desired bacteria.

Characterization of bacterial isolates: The selected colonies were subjected to biochemical characterization using previously described standard methods (Holt *et al.,* 1994). The isolate was molecularly characterized using 16S rRNA sequence analysis. The bacterial genomic DNA was extracted using the phenol–chloroform method, which Sambrook *et al.* described in 1989. (Sambrook *et al.,* 1989). Its quality was assessed using a 1.0% agarose gel, which revealed a single band of high-molecular-weight DNA. The 16S rRNA gene fragment was amplified using the 16S rRNA-F and 16S rRNA-R primers. On an agarose gel, a discrete PCR amplicon band of 1500 bp was observed. To remove contaminants, the PCR amplicon was purified. The PCR amplicon was sequenced forward and reverse using BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer with 16S rRNA-F and 16S rRNA-R primers. Using aligner software, a consensus sequence of the 16S rRNA gene was generated from forward and reverse sequence data. The 16S ribosomal RNA sequences were used to search the NCBI blast search tool for similar sequences. Based on the maximum identity score, closely related sequences were chosen and aligned using the multiple alignment software program Clustal W. A distance matrix and a phylogenetic tree were generated using MEGA 10.

Determination of minimum inhibitory concentration (MIC)
MIC in agar plates: To determine the MIC, the isolates were grown on chromium-infused nutrient agar media**.** The MIC concentration was evaluated by gently inclining the chromium concentration until the isolates failed to form colonies in the Petri plate. The starting concentration of metal was 100 μg/ml from a 100 mg/100 ml stock solution. The streak plate method was used to transfer growing colonies from one concentration to the next higher concentration. After 48 hours of incubation at 37°C, the minimum inhibitory concentration was measured based on the analysis.

MIC in broth: Isolates that exhibited maximum tolerance in agar plates were further subjected to varying chromium concentrations from 100 μg/ml to 1000 μg/ml in broth to confirm their chromium susceptibility. Following incubation, the bacterial growth was estimated with a UV spectrophotometer. For further research, the isolate with the strongest tolerance was carefully selected.

Optimization of *Bacillus subtilis*
For effective chromium degradation, various physicochemical factors, such as pH, temperature, incubation time, inoculum concentration, and glucose percentage, were optimized. The proliferation of the bacterium was analyzed in a UV spectrophotometer, and chromium degradation was assessed by the 1,5 – diphenylcarbazide method (Lace *et al.,* 2019) at 540 nm by spectrophotometry**.**

Chromate bioreduction by free cells of *Bacillus subtilis*: The bacterial strain was cultured in LB broth overnight. Three varying concentrations of Cr(VI) were added to culture flasks containing LB broth medium. (300 μg/ml, 600 μg/ml, 900 μg/ml). Media without Cr(VI) served as a control. The microorganisms were inoculated into these flasks with 0.1 ml of cells under aseptic conditions. The flasks were incubated at 37°C and 120 rpm in a shaking incubator. Samples from each flask were taken at regular intervals (3, 6, 12, 24, and 48 hours), and the biomass of the isolated bacterium was assessed using UV spectrophotometry at OD 600 nm. The reduction of Cr (VI) by growing cells was investigated by taking a 1-ml culture from each flask at the same interval and centrifuging it at 8000 rpm for 10 min. AAS

was used to test the supernatant for Cr(VI) reduction. The pellet was analyzed by FTIR for any functional group changes.

Fourier transform - infrared spectroscopy (FTIR): Infrared spectra were obtained using an FT-IR spectrophotometer (Shimadzu) using KBr pellets. The bacterial pellet was formed by centrifuging the sample at 10,000 rpm for 10 minutes. The dried sample was powdered finely and thoroughly mixed with KBr. It was examined with a spectrophotometer in the 4000 - 400 cm -1 range, and FT - IR analysis of biomass in the availability and nonavailability of metal was carried out to recognize the functional moieties existing in the cell wall of the bacteria that are responsible for biosorption efficiency.

Results and Discussion
Physicochemical and heavy metal analysis of the tannery effluent sample
Tannery wastewater is alkaline (pH 8.1) in nature, light brown in color, and low in dissolved oxygen. In addition, the BOD (biological oxygen demand), TDS (total dissolved solids), and TSS (total suspended solids) were measured to be 550±40 mg/L, 3199 mg/L, and 1989.30 mg/L, respectively. (Table 1). The tannery effluent revealed high TSS and low dissolved oxygen. The suspended particles that settle on the soil can harm soil fauna and cause changes in the porosity of the soil, water-holding capacity, and soil texture (Chowdhury et al., 2013) (Jeyasingh and Philip, 2005). Dissolved oxygen concentrations of 5–8 mg/L are suitable for aquatic environments, and dissolved oxygen concentrations of less than 4 mg/L are considered critical (Akan et al., 2007) (Trivedy and Goel, 1984) (Alam, and Malik, 2008). According to Verma and his colleagues (Verma et al., 2008), the low dissolved oxygen levels (4 mg/L) in the effluents were caused by an increased rate of organic pollution, indicating that the effluents had a high level of BOD. Ten different heavy metal concentrations of the tannery effluent were analyzed by a Shimadzu Atomic Absorption Spectrophotometer (AA-6300) at the Instrumentation Centre, Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi. The tannery effluent sample contained a greater concentration of chromium (0.60 mg/L)

(Table 1). Chromium is highly lethal even in lower quantities, as it has biomagnification properties and accumulates in the food chain, causing toxicity at a cellular level (Barthwal et al., 2008). Continuous discharge of low-concentration chromium is reported to be harmful to aquatic life and disrupts the aquatic food chain (Fent, 2004).

Table 1: Physicochemical characteristics and heavy metal analysis of the tannery effluent

Parameter	Standard Value	Effluent Sample
pH	6-9	8.1
TSS (mg/L)	150-500	1989.30
TDS (mg/L)	2100	3199
BOD (mg/L)	50-250	550±40
Chloride (mg/L)	600	793.3
D.O (mg/L)	4.5-8	1.38
Alkalinity	500	629.7
Temperature	27^0	31°
Heavy metals	**Permissible limit (WHO) mg/L**	**Conc. in mg/L**
Cadmium	0.01	0.0398
Copper	1.5	0.9438
Iron	0.30	0.4013
Chromium	0.05	0.6068
Cobalt	0.01	0.0362
Lead	0.10	0.1606
Magnesium	50	1.4682
Manganese	0.5	0.4497
Nickel	0.1	0.1584
Zinc	5.0	1.3694

Isolation and screening of chromium (Vi) resistant bacterial strains
Five morphologically distinct bacterial strains (C1-C5) were isolated from collected tannery industrial effluent using a serial dilution method, followed by an enrichment culture technique on LB agar plates containing 100 mg/L Cr(VI). Minimum inhibitory concentration screening was performed on all five isolates in both agar plates and broth to determine their resistance to high concentrations of Cr(VI) ranging from 100 to 1000 mg/L. Only one among the five bacterial strains, C1, was identified to tolerate 900 mg/L Cr(VI) in both agar plate and broth culture (Table 2) (Fig. 1). Previous research found that chromium-resistant microorganisms can tolerate concentrations ranging from 100 to 4000 mg/L Cr(VI) (Thacker et al., 2007) (Zhu et al., 2008). The bacterium C1 isolated falls well within the previously described tolerance range. High chromium tolerance bacterial strains were better at

Table 2: Minimum inhibitory concentration of the isolates on nutrient agar plates

S. No.	Test Isolates	Minimal Inhibitory Concentration (µg/ml)									
		100	200	300	400	500	600	700	800	900	1000
1	C1	+	+	+	+	+	+	+	+	+	-
2	C2	+	+	+	+	+	+	-	-	-	-
3	C3	+	+	+	-	-	-	-	-	-	-
4	C4	+	+	+	+	+	+	+	+	-	-
5	C5	+	+	+	+	+	+	+	-	-	-

+ = Growth, - + = No growth

converting Cr(VI) to Cr(III) and can be used to develop a more effective bioagent for chromium bioremediation (Ilias *et al.,* 2011). The C1 bacterial strain was chosen for further Cr(VI) reduction studies based on the results of the above screening.
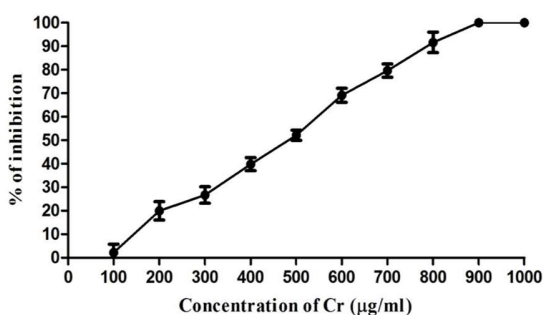
Morphological, biochemical, and molecular characteristics of isolated chromium (Vi) resistant bacterium

Bacterial isolate C1 was identified as gram-positive, rod-shaped, motile, endospore-forming bacteria producing white colonies on nutrient agar medium. Biochemical tests were performed for the characterization of bacteria using Bergey's Manual of Determinative bacteriology (Holt *et al.,* 1994). Table 3 summarizes the morphological and biochemical analysis of the isolated bacterium (C1). This bacterium showed positive reactions for catalase, Voges Proskauer, citrate, gelatin hydrolysis, starch hydrolysis, and nitrate reductase tests but negative responses for the indole and

Figure 1: Minimum Inhibitory Concentration of Isolate C1 in Broth

methyl red tests. The TSI slants were alkaline, and they fermented only glucose but could not ferment lactose and sucrose. Furthermore, 16S rRNA gene sequence findings indicate that the isolated bacterium (C1) was 99.6% similar to that of the genus *Bacillus*, and thus, based on sequence similarity and blast analysis, the isolated bacterium (C1) was identified as *Bacillus subtilis* (Figure 2) with accession number MW737447. *Bacillus* has a widespread distribution and has adapted to harsh environments. (Arahal *et al.,* 2002). The spore-bearing distinctive feature most likely confers high Cr(VI) resistance to this Bacillus strain and allows it to remain in the stationary phase for an extended period of time. (Zouboulis *et al.,* 2004).

Optimization of chromium tolerant *Bacillus subtilis*

The proliferation of bacteria and simultaneous deduction of Cr(VI) by *Bacillus subtilis* C1 was assessed by subjecting it to various parameters, such as pH, temperature, incubation time, inoculum concentration, and glucose percentage, to optimize the environmental factors for better chromium degradation. Environmental variables such as pH, temperature, concentration, and the length of the bioreduction process all have a significant impact on the bioremediation process (Selvi *et al.,* 2014) (Sathishkumar *et al.,* 2017).According to the findings of this study, *Bacillus* subtilis C1 exhibited maximum cell growth and reduction efficiency of chromium at pH 7.0 (Fig. 3), a temperature of 30°C (Fig. 4), glucose 2% (Fig. 5), inoculum concentration of 6% (Fig. 7) and incubation time of approximately 24 hrs. (Fig. 6). Previous research has shown that weakly acidic conditions are more favorable for Cr6+ removal (Liu *et al.,* 2020). Similarly, when the pH was greater than 8 or slightly less than 5, the interaction of the Cr6+ removal enzyme synthesized by Bacillus subtilis was altered, which led to a substantial decrease in Cr6+ bioreduction. (Dhal *et al.,* 2010). Following a24-hour growth phase, the microbial community enters a death phase, and the microbial activity will decrease (Rolfe *et al.,* 2012). Temperature plays a
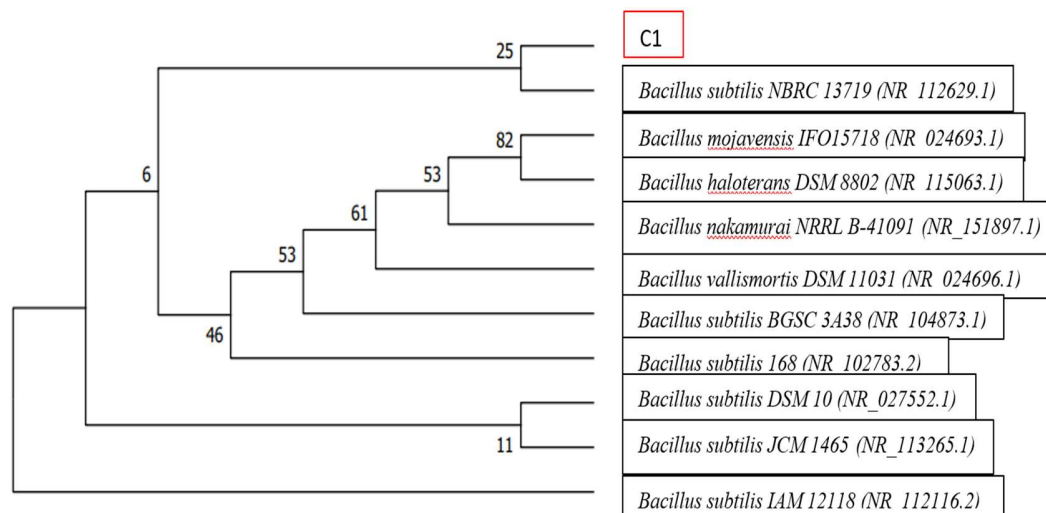
Figure 2. Molecular phylogenetic analysis of *Bacillus subtilis C1* by maximum likelihood. Accession numbers are indicated after the name of the isolate
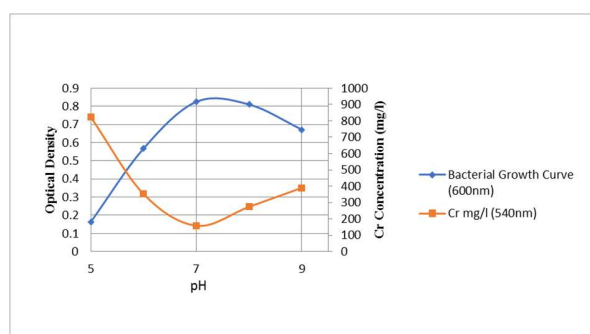

Figure 3: The effect of pH on chromium degradation by *Bacillus subtilis* C1
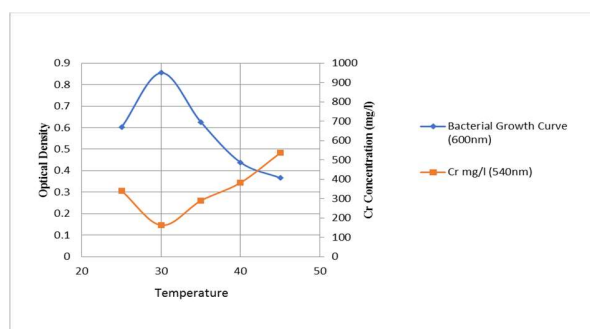

Figure 4: The effect of temperature on chromium degradation by *Bacillus subtilis* C1. Error bars indicate the standard deviations.

critical role in bacterial growth and enzyme activity, and the optimal temperature for both B. cereus D and B. cereus 322 growth is 30°C (Li *et al.,* 2020). Glucose, fructose, lactose, pyruvate, lactate, citrate, glycerol, acetate, formate, NADH/NADPH, and reduced glutathione are well-known electron donors for Cr(VI) reduction (Murugavelh,& Mohanty,2013) (Mala *et al.,* 2015). Because glucose is the easiest carbon source to metabolize, it is capable of offering most electrons for Cr(VI) reduction (Zheng *et al.,* 201).

**Reduction of chromium (Vi) by free cell of *Bacillus subtilis***

The flask culture method of *Bacillus subtilis* C1 for Cr(VI) reducing capability was monitored in LB broth medium at 300, 600, and 900 µg/mL Cr(VI) at five different intervals of 3, 6, 12, 24, and 48 hours under aerobic conditions. In our experiment, a maximum Cr(VI) reduction of approximately 78% was discovered in 300 µg/mL Cr(VI) in 24 hours, confirming the exponential bacterial cell growth phase. At high concentrations of 600 µg/mL and 900 µg/mL, the chromium was reduced to 70% and 50%, respectively (Figure 8). The bacterium *Bacillus subtilis* C1 alleviated Cr(VI) in a concentration-dependent manner, which meant that the bacterium's removal efficiencies decreased as the Cr(VI) concentration increased. When the Cr(VI) concentration ranged from 300 to 900 g/ml, more than 50% of the Cr(VI) was reduced in 24 hours. However, after 48 hours of incubation, the reduction at 900 g/ml was only 40%. The bacterium's lower chromium reduction potential at

Table 3: Morphological and biochemical characteristics of the bacterial isolate _Bacillus subtilis_ (_C1_)

S.No	Characteristics	_Bacillus subtilis (C1)_
1	Cell Shape	Rod
2	Color	white
3	Gram's staining	+
4	Motility	+
5	Spore formation	+
6	Indole	-
7	Methyl red	-
8	VP	+
9	Citrate utilization	+
10	Triple sugar agar test	K/A
11	H2S production	-
12	Starch hydrolysis	+
13	Gelatin hydrolysis	+
14	Catalase	+
15	Nitrate reductase	+

+ = Positive, - = Negative
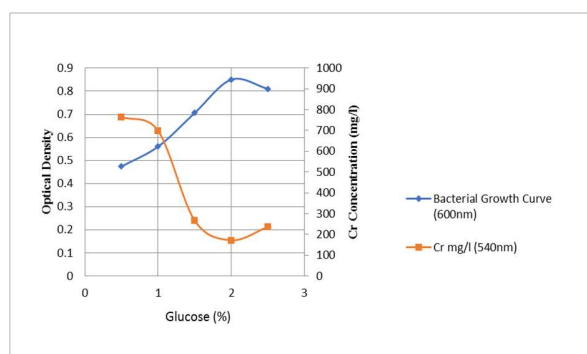
Figure 5: The effect of glucose (%) on chromium degradation by _Bacillus subtilis_ C1. Error bars indicate the standard deviations
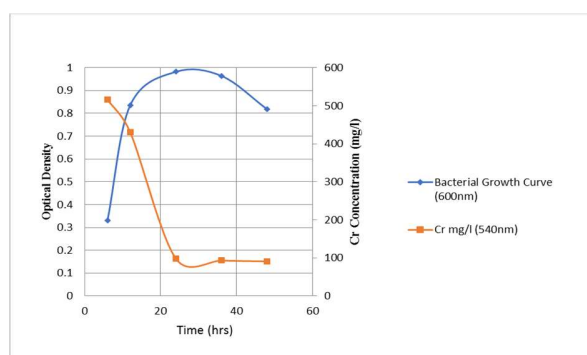
Figure 6: The effect of time on chromium degradation by _Bacillus subtilis_ C1. Error bars indicate the standard deviations

elevated concentrations may indeed be related to Cr(VI) mutagenic and toxic effects on bacterial cell metabolism. (Thacker _et al.,_ 2007). Because of hexavalent chromium's inhibitory action, bacterial cell density decreases slightly as concentration increases (Xiao _et al.,_ 2017). In previous studies, _Bacillus sp._ is known to be resistant up to 40 mg/L and decreases thereafter. This indicates that as the concentration increases, the growth of _Bacillus sp._ is inhibited. _Bacillus._ isolated by Murugavelh and Mohanty removed 96.8% of Cr(VI) in 48 h with a 1.15 mM initial Cr(VI) concentration (Murugavelh & Mohanty, 2013). It has also been reported earlier that after 120 hours of incubation, Alcaligenes faecalis was reduced by approximately 70.0% and Bacillus sp. (accession number FM208185.1) was reduced by only 73.41% of 100 mg/L Cr(VI) (Sun _et al.,_ 2018). When the bacterial growth curve reaches a certain point, it appears saturated, indicating adaptive mechanisms that enable the isolate to confer resistance to toxic Cr(VI) and grow in its presence (Cervantes & Campos-García, 2007)

FTIR analysis

FTIR spectroscopy was employed to determine the properties of the functional groups and chemical bonds that were essential in the biosorption of hexavalent chromium. The FTIR spectra of chromium-treated and untreated biomass revealed some absorption bands at different wavelengths, indicating the presence of a number of functional groups, such as amine, carboxyl, amide, aliphatic, aromatic groups, and bonded and unbonded hydroxyl groups. The biomass was treated with three concentrations of chromium (300, 600, and 900 µg/ml), and the IR spectra were compared. Absorption peaks differed slightly between chromium-treated and untreated biomass. The untreated bacterial biomass revealed peaks at 1067. 53 cm-1, 1643.24 cm-1, 2041.51 cm-1, and 3524.67 cm-1 correspond to C-N, -C = C-, N=C=S and (-COOH) bonds, respectively. (Figure 9). These bonds suggest the involvement of various functional moieties, such as amides and carbohydrates, on the outer surface of Bacillus sp. in the metal adsorption process (Bağcıoğlu _et al.,_ 2019). The FT-IR spectra of metal-loaded bacteria showed a highly significant shift in frequency from 3524.67 cm-1 to 3489.95 cm-1 (300 µg/ml) (Figure 10), 3444.63 cm-1 (600 µg/ml) (Figure 11) and 3444.63 cm-1 (900 µg/ml) (Figure 12), suggesting the strong NH2 asymmetric stretching mode of
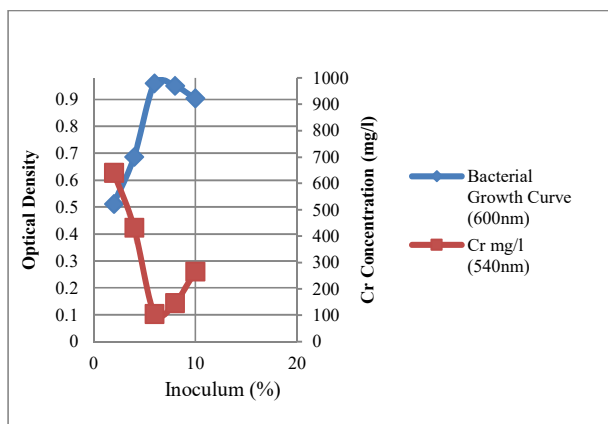
Figure 7: The effect of inoculum (%) on chromium degradation by *Bacillus subtilis C1*. Error bars indicate the standard deviations
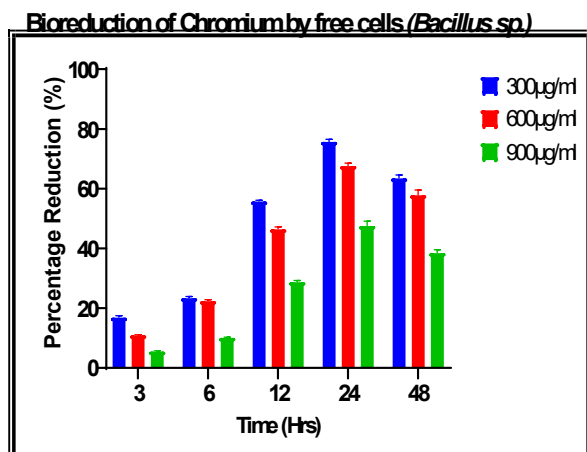

Figure 10: FTIR analysis of chromium (300 μg/ml)-treated cells of *Bacillus subtilis C1*

Figure 8: Bioreduction of chromium by free cells of *Bacillus subtilis C1*

Figure 11: FTIR analysis of chromium (600 μg/ml)-treated cells of *Bacillus subtilis C1*
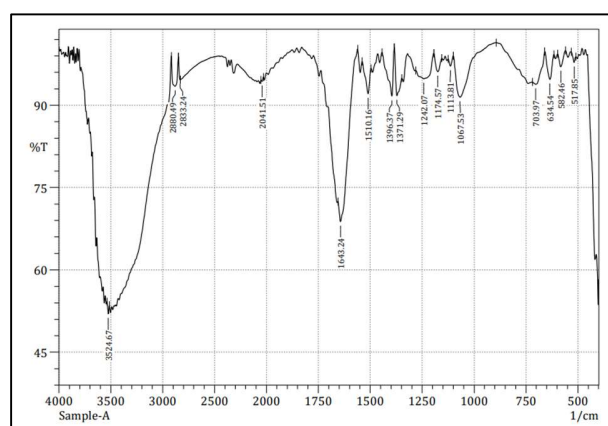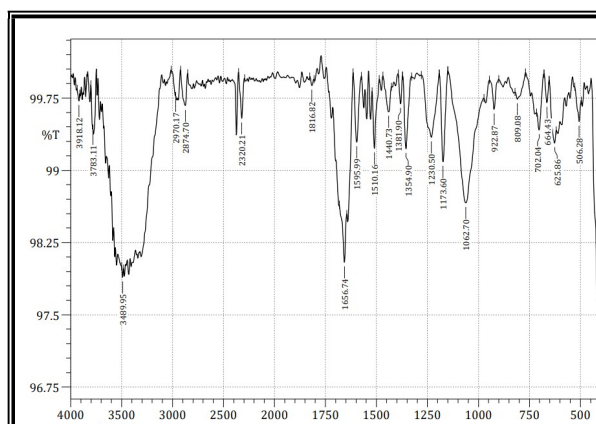

Figure 9: FTIR analysis of untreated cells of *Bacillus subtilis C1*

Figure 12: FTIR analysis of chromium (900 μg/ml)-treated cells of *Bacillus subtilis C1*
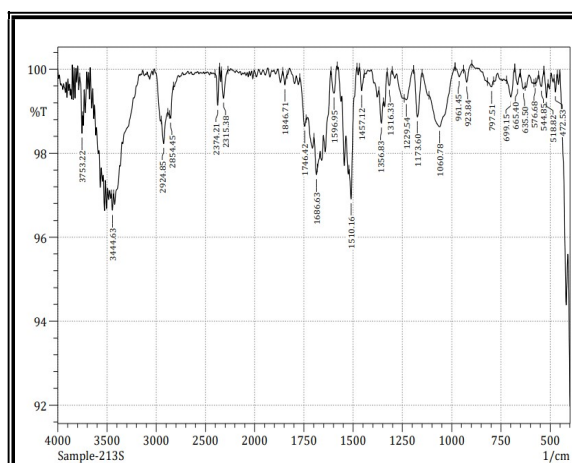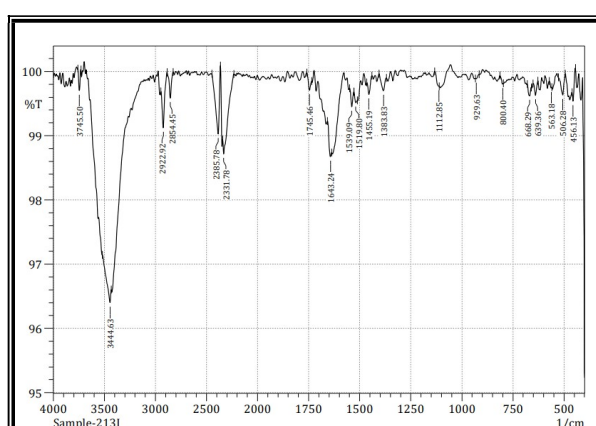
amines, which indicates the overlapping of amines and hydroxyl stretching on the surface of the bacterial cell (Mungasavalli *et al.,* 2007). Similarly, 1643.24 cm^{-1} (C=C stretching) of untreated biomass shifted to 1656.74 cm^{-1} (C=C stretching) in the 300 µg/ml chromium-loaded sample, 1686.63 cm^{-1} (C=N stretching) in the 600 µg/ml sample and 1643.24 cm^{-1} (C=C stretching) in the 900 µg/ml chromium-loaded sample. The slight shift from 1643 cm^{-1} to a higher frequency region of 1650-1680 cm^{-1} indicated the intervention of the C=O group of the amide I bond stretching (Doshi *et al.,* 2007). The peak at 1510 cm^{-1} remains constant in both treated and untreated biomass and is attributed to the aromatic ring's C-C stretching vibration, which indicates the presence of aromatic CH bending vibrations (Pham *et al.,* 2016). Similarly, the absorption peak that appeared at 2967 cm–1 remained unchanged in regard to both Cr(VI)-exposed and -unexposed cells. It might be said that the complexation of Cr(VI) with hydroxyl, carbonyl, or amide moieties causes reduction, more specifically between the wavelengths of 3400 and 3550 cm-1 and 1600 and 1750 cm-1. (Shahadat *et al.,* 2015).

Conclusion
The important findings of this paper were that the isolated chromium-tolerant bacterium *Bacillus*

subtilis C1 had a higher Cr(VI) reducing power and could reduce up to 78% of Cr(VI)when compared to other isolated bacterial species. *Bacillus subtilis* C1 also exhibited the highest MIC value of 900 mg/L Cr(VI) and the spore-forming capability of *Bacillus* isolates gives an added advantage of lyophilizing the sporulating biomass and utilizing it in the biological clean-up of chromium-contaminated sites and the biological treatment of wastewater. These discoveries can be transcribed into technology through small-scale pilot testing and, sooner or later, on a massive scale to clean up chrome-polluted wastes, including tannery effluents, before they are discharged into the environment.

Acknowledgement

Conflict of interest
The authors declare that they have no conflict of interest.

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