



Staphylococcus edaphicus KCB02A11 incorporated with natural adsorbents: first report on its tolerance and removal of hexavalent chromium [Cr(VI)]

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Abstract

Deteriorating the quality of different parts of the ecosystem due to toxic metals is a serious global issue. Hexavalent chromium is a metal that can cause adverse effects on all living beings, including plants, animals, and microorganisms, on exposure to high concentrations for prolonged periods. Removing hexavalent chromium from various types of wastes is challenging; hence the present study investigated the use of bacteria incorporated with selected natural substrates in removing hexavalent chromium from water. Isolated *Staphylococcus edaphicus* KCB02A11 has shown higher removal efficiency with a wide hexavalent chromium range (0.025–8.5 mg/L) within 96 h. Incorporating the isolated strain with natural substrates commonly found in the environment (hay and wood husk) showed high removal potential [100% removal with 8.5 mg/L of Cr(VI)], even within less than 72 h, with the formation of biofilms on the used substrates applied for metal removal on a large scale for prolonged periods. This study is the first report investigating hexavalent chromium tolerance and removal by *Staphylococcus edaphicus* KCB02A11.

Keywords Adsorbent · Hexavalent chromium (Cr(VI)) · Biofilms · *Staphylococcus edaphicus*

Introduction

Heavy metals are essential raw materials in many industrial applications and inevitably generate a significant amount of metal-contaminated wastes. Environmental pollution caused by heavy metal accumulation in the ecosystem has become a global concern, where adverse effects are rendered on flora and fauna due to toxicity and subsequent bioaccumulation, persistency, and least potential of biodegradability (Mehmood et al. 2019; Ravindra and Mor 2019; Ukah et al. 2019; Kaur and Roy 2021).

Chromium (Cr) compounds are widely used in leather tanning, pigment and dye production, electroplating, wood preserving, and refractory materials (Almeida et al. 2019; Coetzee et al. 2020). Of two of the common forms of Cr, trivalent chromium [Cr(III)] is known as naturally occurring in nature, while hexavalent chromium [Cr(VI)] is produced by the industrial process (Owlad et al. 2009). Furthermore, it is a known fact that Cr(VI) is highly toxic than Cr(III) to living beings as it is highly soluble and mobile (Megharaj et al. 2003; Pan et al. 2014; Ertani et al. 2017).

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Prolonged exposure to Cr(VI) may cause kidney and liver dysfunctions, nasal irritations, nasal perforations, skin irritations, skin ulcerations, skin allergies and lung cancers in animals, including humans (Kotaś and Stasicka 2000; Owlad et al. 2009). Based on this sufficient evidence, International Agency for Research on Cancer (IARC) announced that Cr(VI) as a carcinogenic agent in 1990 (Saha et al. 2011). Furthermore, exposure to Cr compounds can cause toxic effects on higher plants at the level of $100 \mu\text{M Kg}^{-1}$ on a dry weight basis (Davies et al. 2002). Besides, chromium toxicity can reduce germination, growth pattern changes, reduction of yield, and increase seed deforming, etc. in plants while leading to growth inhibitions, lag phase elongations, inhibition of oxygen uptake, denaturing enzymes and mutagenesis on microorganisms (Cervantes et al. 2001; Shanker et al. 2005; Igiri et al. 2018).

Due to the toxicity, international and national legislation authorities have regulated Cr(VI) disposal. World Health Organization (WHO) and the United States Environmental Protection Agency (US EPA) have mainly concerned with drinking water quality and recommend a maximum permissible level for Cr(VI) of 0.05 mg/L (Altun et al. 2016; Cherdchoo et al. 2019). Concerning the higher exposure probability, Occupational Safety and Health Administration (OSHA) has legislated the maximum exposure limit for Cr(VI) compounds for 8-hour work shifts and 40-hour workweeks as 0.052 mg/L to the occupational community (Mishra and Bharagava 2016). However, these discharge limits change according to national and regional standards, the type of industry, and the nature of the discharge environment (Vaipoulou et al., 2020).

Therefore, Cr(VI) contaminated effluents need to be remediated into less toxic and less soluble forms to minimize harmful effects on the ecosystem, and is possible to achieve physical, chemical and/or biological methods. The Physical remediation can be done with techniques such as adsorption, electrolysis, membrane filtration, soil washing and capping (Jobby et al. 2018; Shahnaz et al. 2020).

Chemical remediation is mainly based on the reduction of Cr(VI) into Cr(III) using a variety of chemicals such as SO_2 , CaS_5 , $\text{Na}_2\text{S}_2\text{O}_5$, Na_2SO_3 , FeSO_4 , BaSO_3 , TiO_2 , N_2H_4 , H_2O_2 , and Ca(OH)_2 (Ma et al. 2016; Dong et al. 2018; Jobby et al. 2018). On the contrary, bioremediation is functioned with bacteria, fungi, algae and plants with low energy consumption, low operational costs, fewer environmental health hazards, and with high efficiency compared to physical and chemical methods (Fernández et al. 2013; Narayanasamy et al. 2022).

Several bacterial species such as *Staphylococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Deinococcus* sp., *Shewanella* sp., *Agrobacterium* sp., *Escherichia* sp., *Thermus* sp., *Microbacterium* sp., *Desulfovibrio* sp., *Deinococcus*

sp., *Brucella* sp., and *Bacillus* sp. were identified as Cr(VI) removers from contaminated sources through “directly” with enzymes or “indirectly” with metabolic end products (Vatsouria et al. 2005; Mistry et al. 2010; Mythili and Karthikeyan 2011; Sharma and Adholeya 2012; Thatoi et al. 2014; Suresh et al. 2021). Furthermore, Narayani & Shetty (2013) and Igiri et al. (2018) report that microbial bioremediation of Cr(VI) can be stimulated or inhibited by physical and chemical factors, including pH value, cell density, initial Cr(VI) concentration, temperature, aerobic/anaerobic environment, electron donors, oxyanions, salt concentration, presence of heavy metals, metabolic inhibitors and oxidation-reduction potential etc.

Representatives of the *Staphylococcus* genus, such as *S. epidermidis*, *S. sciuri*, *S. cohini*, *S. aureus*, and *S. xylocus*, etc., have shown Cr(VI) removal potential with different efficiencies (Table 1). However, the Cr(VI) tolerance and removal potential of *Staphylococcus edaphicus* has not previously been reported. The current study reports the potential of isolated *Staphylococcus edaphicus* and its biofilms incorporated with natural adsorbents in Cr(VI) removal.

Methods

Identification of bacteria

The bacterial strain isolated previously from a study carried out by Seneviratne & Rathnayake (2019) was used in this study. The DNA extraction, 16 S rRNA gene amplification and PCR product sequencing were done at Genetech Molecular Diagnostics and School of Gene Technology, Sri Lanka. Bacterial 16 S rRNA gene sequence was amplified by Polymerase chain reaction (PCR) using forward and reverse universal primers [785 F (5'-GGATTAGATACCCTGGTA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3')] while post molecular analysis was followed with NCBI GenBank database and relevant open-source software. The tasks of the chromatogram sequence edition process, forward and reverse sequence aligning process, and consensus sequence preparations were completed using BioEdit Sequence Alignment Editor (Version 7.2.5). The obtained bacterial DNA sequence was deposited in the NCBI GenBank with all relevant data. The phylogenetic relationship of the bacterial isolate was determined through phylogenetic tree construction using Mega 10.2.6.

Preparation of metal stock solutions and chemical analysis

Cr(VI) stock solution (100 mg/L) and working Cr(VI) solutions were prepared by dissolving appropriate amounts

Table 1 Cr(VI) removal potential of several *Staphylococcus* spp

Strain	Tested source	Initial Cr(VI) concentration ^{a,b}	Cr(VI) removal percentage (%)	Time (hrs)	Reference
<i>Staphylococcus cohnii</i>	Tannery wastewater	100 mg/L	90	96	(Saxena et al. 2000)
<i>Staphylococcus epidermidis</i> HJ2	Synthetic	25 mg/L	76.8	168	(He et al. 2019)
<i>Staphylococcus xylosus</i>	Synthetics	50 mg/L	78.3	24	(Aryal et al. 2011)
<i>Staphylococcus epidermidis</i> L-02	Synthetic	0.3 m mol	100	72	(Vatsouria et al. 2005)
<i>Staphylococcus sciuri</i> A-HS1	Tannery waste	2 mM	93	144	(Elahi et al., 2019)
<i>Staphylococcus aureus</i> LZ-01	Synthetic	0.4 mM	66.5	53	(Zhang et al., 2014)
<i>Staphylococcus saprophyticus</i>	Synthetic	50 µg mL ⁻¹	100	240	(Pereira et al., 2019)
<i>Staphylococcus arlettae</i>	Synthetic	50 µg mL ⁻¹	100	240	(Pereira et al., 2019)
<i>Staphylococcus xylosus</i>	Spiked soil	20 µg mL ⁻¹	100	240	(Pereira et al. 2017)
<i>Staphylococcus gallinarum</i>	Spiked soil	20 µg mL ⁻¹	100	240	(Pereira et al. 2017)
<i>Staphylococcus aureus</i>	Synthetic	40 mg/L	68.4	08	(Wang et al. 2020)
<i>Staphylococcus sciuri</i>	Contaminated soil	6.2 mg/Kg	71.0	1344	(Dutta et al. 2017)
<i>Staphylococcus xylosus</i>	Synthetic	400 mg/L	40.0	04	(Ziagova et al. 2007)
<i>Staphylococcus saprophyticus</i>	Synthetic	50 mg/L	100	240	(Pereira et al., 2019)
		20 mg/L	100	96	
<i>Staphylococcus arlettae</i>	Synthetic	50 mg/L	100	240	(Pereira et al., 2019)
		20 mg/L	100	96	

a- Original value and unit by the authors.

b- Converted value into mg/L.

of $K_2Cr_2O_7$ (AnalaR NORMAPUR, Belgium) in deionized water. Analytical verification of metal solutions were assured using inductively coupled plasma mass spectrometry (ICP-MS) according to the method APHA 3120 B: 2017. Furthermore, metal binding in glassware was avoided by acid washing them (with HNO_3) before use.

Tolerance and removal of Cr(VI) by bacteria

Bacterial culture (approximately 10^7 CFU/mL, overnight grown in nutrient broth) was inoculated into Tris minimal (modified) medium (at pH 7) supplemented with a range of concentrations (0.025–16.0 mg/L) of Cr(VI) in the presence and absence of the selected natural adsorbents. Controls were set as media without Cr(VI) but inoculated with the bacterial inoculum. The inoculated bioassay samples were incubated for 96 h., at 100 rpm at room temperature in a shaking incubator (JSSI-202 C Series). Optical density measurements were taken at 600 nm at 24 h. intervals to monitor the growth response of the bacteria during the study period using Thermo Scientific™ Multiskan™ FC Microplate Photometer. The viability of the bacterial isolate was determined during the study period by streaking on nutrient agar solid media at every 24 h. interval. Experiments were carried out in triplicate for a precise study.

Bioassay samples withdrawn at 24 h. intervals were centrifuged at 6000 rpm for 10 min to separate cell-free supernatant and bacterial cells. The pellets were resuspended in a solution (pH 7.0) containing 1×10^{-4} M EDTA with

6×10^{-4} M $CaCl_2$ to adjust the ionic strength and remove absorbed ions from cell surfaces. The resuspended samples were incubated in the dark for 10 min and centrifuged. The supernatants were removed using suction, and cell pellets were washed twice in deionized water to remove further bound ions to cell walls (Miranda and Rojas 2006). Collected cell pellets were acid digested with 2 mL of concentrated HNO_3 acid for 48 h. and reconstituted to 20 mL with deionised water (Madhaiyan et al. 2007).

Initial Cr(VI) amount added to the test solution, including the remaining Cr(VI) amount in cell-free supernatant and acid-digested bacterial cells were quantified using US EPA Method 7196 A with 1,5-diphenyl carbazide (DPC) (Megharaj et al. 2003; Lee et al. 2003), and analytically verified using ICP-MS as per the APHA 3120 B: 2017 protocol.

Analysis of Cr(VI) removal potential by selected adsorbents

Dried hay and wood husk (10.0 g/L) were cut into irregular lengths between 5 and 10 cm and sterilized by autoclaving. Assays were conducted in the presence of Cr(VI) but without the bacterial culture as described earlier. Initial Cr(VI) and remaining Cr(VI) content after 96 h. were measured using the DPC method every 24 h. during the experiment.

Detection of bacterial biofilm formation

Bacterial culture was inoculated into Tris minimal (modified) medium and incubated at 37 °C for 18 h. Incubated culture (1 mL) was added into the freshly prepared same medium with a 1:100 ratio. Aliquots of 0.2 mL were transferred into 96 well, flat-bottomed polystyrene sterile microtiter plate and incubated at 37 °C for 24 h. After the incubation period, contents in the microplate wells were gently removed by an aspirator and washed four times using phosphate-buffered saline (PBS pH 7.2) (0.20 mL). Wells were filled with sodium acetate (2%, 0.20 mL) and stained with crystal violet (0.1% w/v) for 5 min. Excess stains were removed and rinsed off using deionized water. Plates were air-dried, and the optical density of stained cell adhered wells were measured at 570 nm (Christensen et al. 1985; Mathur et al. 2006).

Bacterial biofilm formation on the adsorbents and their Cr(VI) removal potential

Pre-sterilized adsorbents and the bacterial culture were added (approximately 10^7 CFU/mL, grown overnight in nutrient broth) to Tris minimal (modified) medium and allowed to form biofilms on adsorbents for 48 h., at 100 rpm at room temperature in the shaking incubator. The bioassay samples were supplemented with Cr(VI) (8.5 mg/L) and incubated for another 96 h. at the same growth conditions. The concentration of Cr(VI) at 8.5 mg/L was selected based on the removal performance of the selected bacterial strain during the experimental period. Cr(VI) content of the bioassay samples was measured by the DPC method at every 24 h. interval up to 96 h. using the cell-free supernatant prepared by centrifugation. The bacterial biofilm formation was also visualized by ZEISS Scanning Electron Microscope (AT 10.00 KV).

Estimation of Cr(VI) removal

Cr(VI) removal of the bacterial strain, adsorbent, and strain incorporated adsorbents (biofilms) were calculated as a percentage using mathematical expression based on data collected by DPC colorimetric method as described in Krishna and Sree (2013).

Statistical analysis

The medium effective concentrations (EC_{50}) of bacterial isolate and Cr(VI) removal potential of natural adsorbents, bacterial isolate and their biofilms were calculated by probit analysis and independent samples Kruskal – Wallis test

respectively with 95% confidence using IBM SPSS Statistics, version 25.

Results

Molecular identification of the bacterial isolate.

The aligned 16 S rRNA consensus sequence of the bacterial isolate was identified with NCBI's BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Kabir et al. 2018; Pradhan et al. 2020). According to the blasting suite, the isolated strain exhibited higher similarity to *Staphylococcus edaphicus* CCM 8730 (Accession No. NR156818.1) with 100% query cover, 0.0 of E-value and 99.88% of percentage identity. Moreover, the phylogeny of the isolate revealed a higher phylogenetic relationship with *Staphylococcus edaphicus* strains that were aligned using the MUSCLE algorithm and the constructed phylogenetic tree using MEGA 10.2.6 (Fig. 1). The bacterial DNA sequences obtained in this study were deposited in the GenBank database under the accession number OL881276. The identified bacterium was named *S. edaphicus* KCB02A11 here in.

Growth response of *S. edaphicus* KCB02A11 in Cr(VI) supplemented media

According to dose-response bioassays, *S. edaphicus* KCB02A11 has shown higher tolerance up to 16.0 mg/L of Cr(VI). The growth response curves of *S. edaphicus* KCB02A11 exhibited a gradual growth in all tested Cr(VI) contents except the highest tested concentration of 16.0 mg/L which showed a slight growth compared to others. However, none of the concentrations has surpassed the bacterial growth in non-metal added control (Fig. 2). This change of patterns may be due to the toxicity of Cr(VI) towards bacteria. According to the dose-response analysis at 600 nm high bacterial growth was observed under low Cr(VI) contents while lower growth was in high Cr(VI) concentrations.

The 50% effective concentration (EC_{50}) estimations (Table 2) of the bacterial strain resulted in different values for every 24 h. time interval, decreasing with exposure time up to 96 h. in considerable amounts from 78.686 mg/L (in 24 h.) to 0.084 mg/L (in 96 h.) This may be due to the toxicity of Cr(VI) towards bacterial cells with time.

Total chromium removal by *S. edaphicus* KCB02A11

Cr(VI) removal studies showed that it was evident that the *S. edaphicus* KCB02A11 could achieve a complete Cr(VI) removal up to the concentration of 8.5 mg/L within the

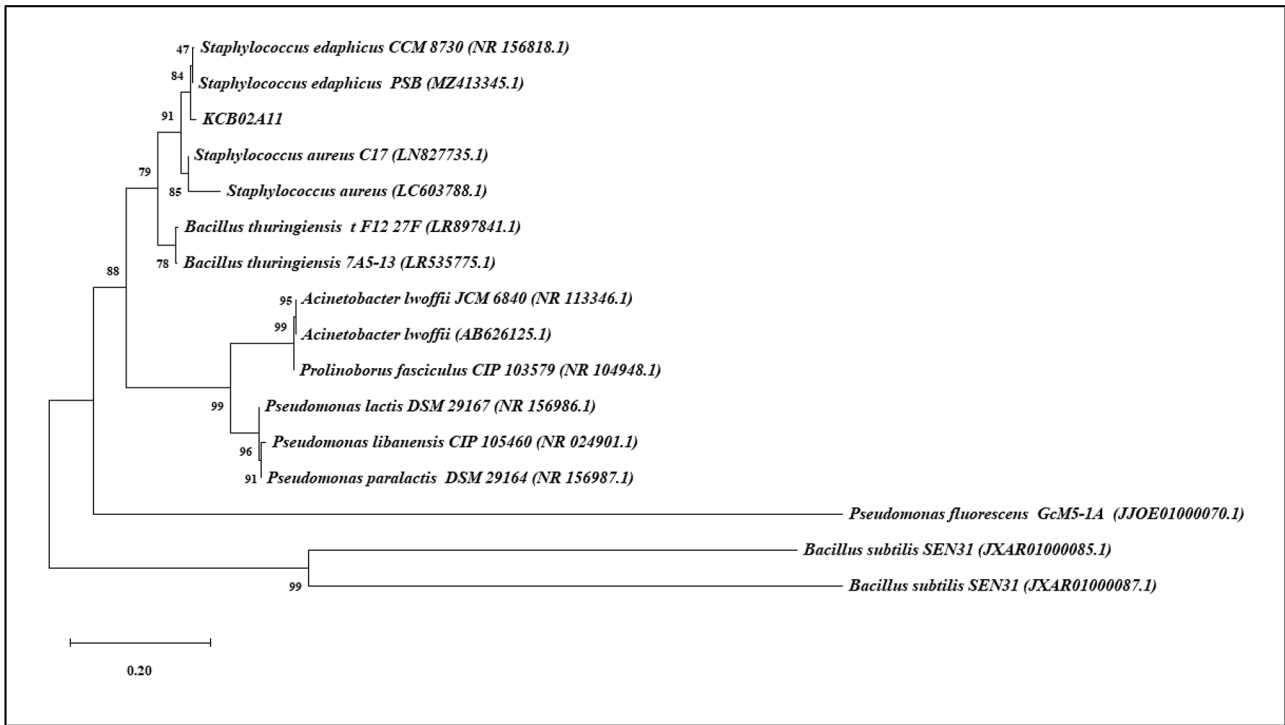


Fig. 1 A neighbour-joining phylogenetic tree based on 16 S rRNA gene sequences showing the phylogenetic relationship among isolated strain *S. edaphicus* KCB02A11 and other selected species constructed using

Mega 10.2.6. The bootstrap numbers indicate the value of 1000 replicate trees. The NCBI accession numbers are given in parenthesis

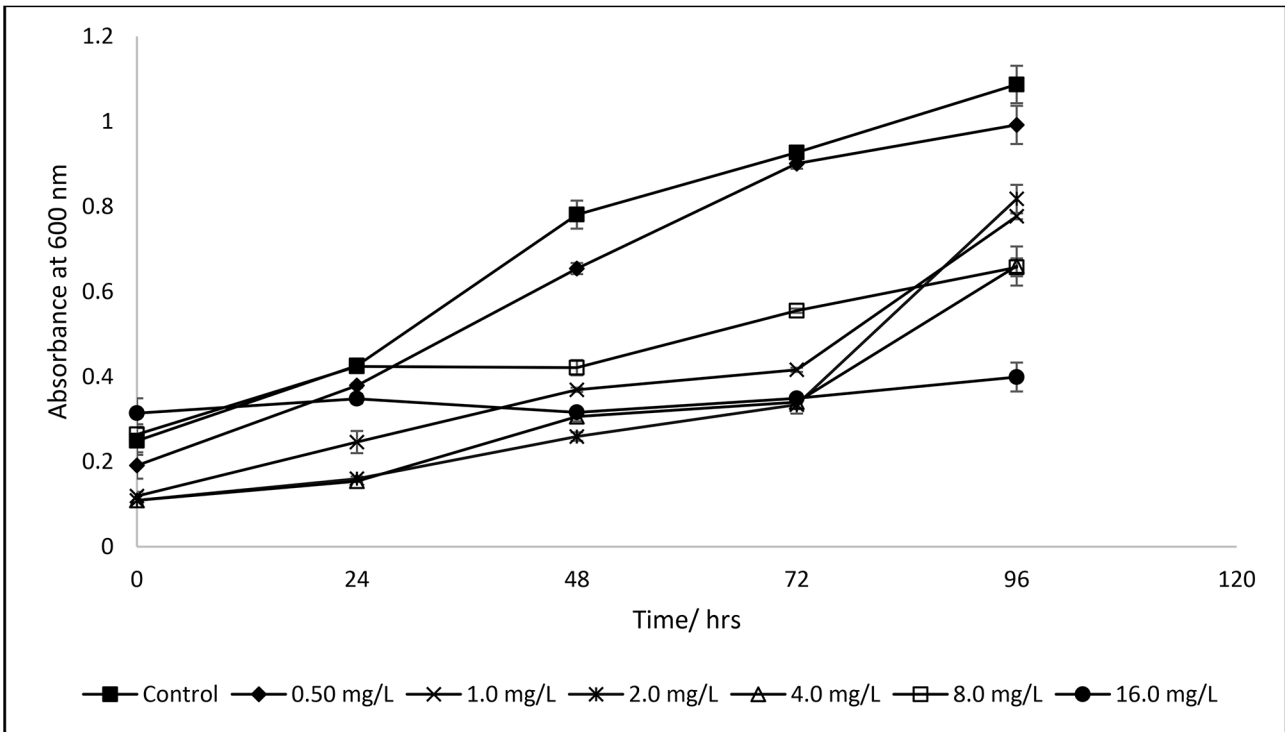


Fig. 2 *S. edaphicus* KCB02A11 growth response in different Cr(VI) concentrations. (Error bars represent standard deviation 0.002–0.046)

Table 2 Variance of EC₅₀ values of *S. edaphicus* KCB02A11 with 95% confidence level under 0.025–16.0 mg/L of Cr(VI).

Time (hrs.)	EC ₅₀ value
24	14.824
48	0.088
72	0.021
96	0.000

study period of 96 h. following a gradual decrease of Cr(VI) removal in higher concentrations (Fig. 3A and B).

The graphical interpretations further revealed a complete Cr(VI) removal by bacterial cells in suspension within 24 h. at lower concentrations (0.025–0.5 mg/L) while complete

removal of 8.5 mg/L within 96 h. required a gradual increase in time. Removal potential of the tested concentrations higher than 8.5 mg/L showed the same pattern but around 50% maximum removal at the end of the experimental period (96 h). This analysis also revealed a negative correlation between the Cr(VI) removal potential of the bacterial isolate and the metal concentration as it exhibited a decrease of Cr(VI) removal at 8.5 mg/L of Cr(VI) and afterwards (Fig. 3A, and Fig. 3B). Moreover, the results of the Cr(VI) analysis of acid digested cell pellets also indicate the absence of Cr(VI) inside and cell walls of bacterial cells.

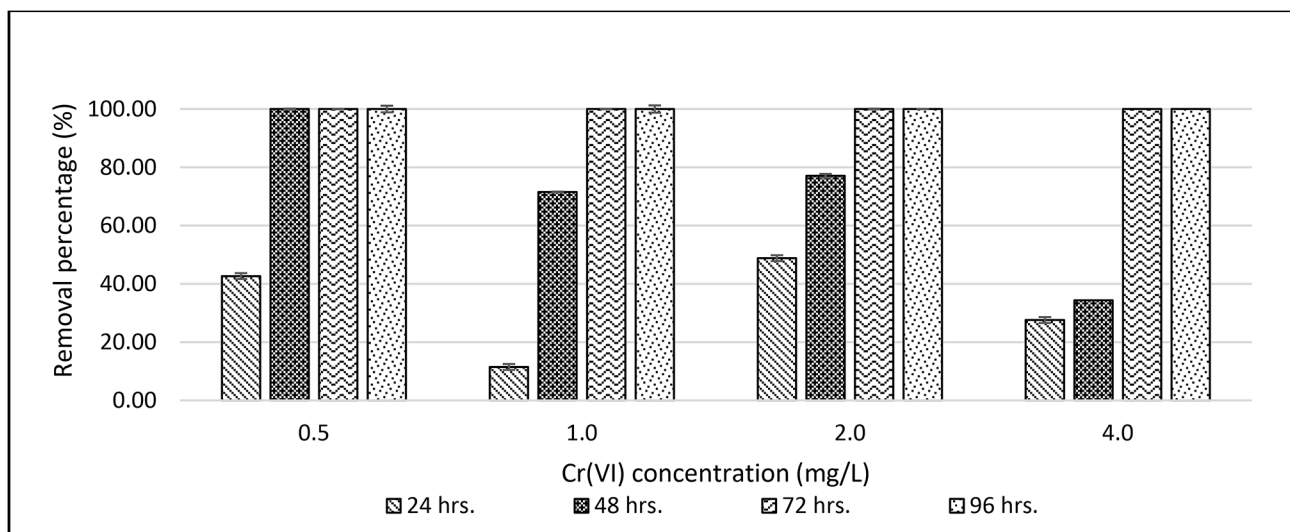


Fig. 3A Cr(VI) removal by *S. edaphicus* KCB02A11 in low Cr(VI) concentrations (Error bars represent standard deviation 0.06–1.13)

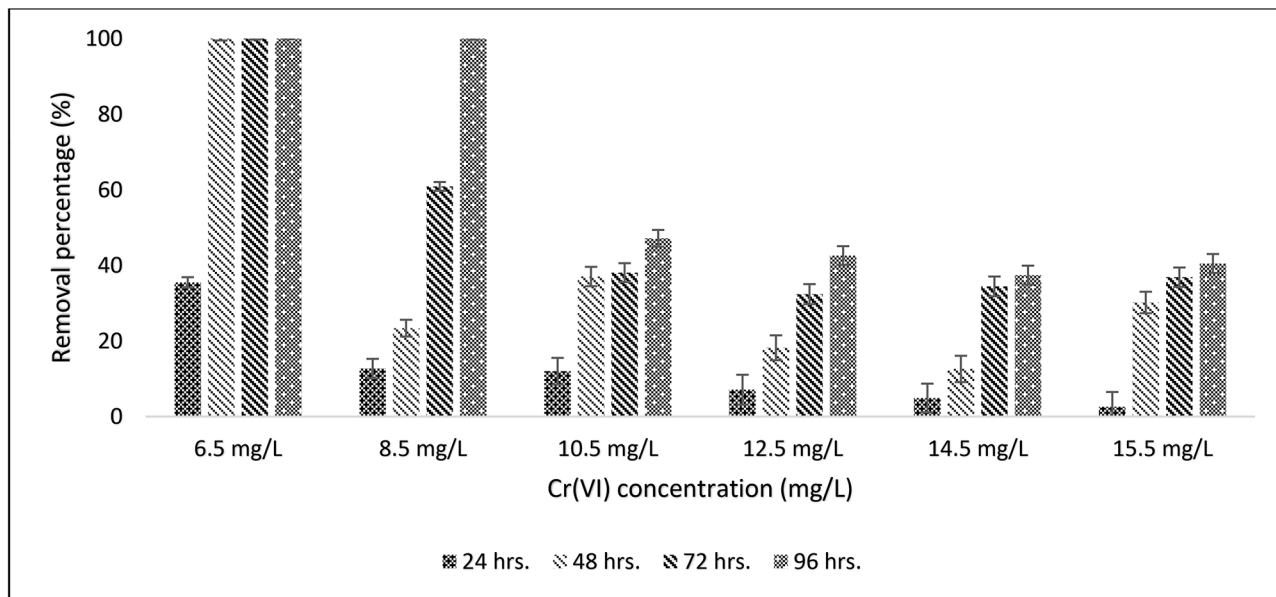


Fig. 3B Cr(VI) removal by *S. edaphicus* KCB02A11 in high Cr(VI) concentrations (Error bars represent standard deviation 0.187–3.987)

Fig. 4 Removal of total chromium by *S. edaphicus* KCB02A11 at the end of 96 h. (with standard deviation percentile at 95% confidence (1.26–2.11))

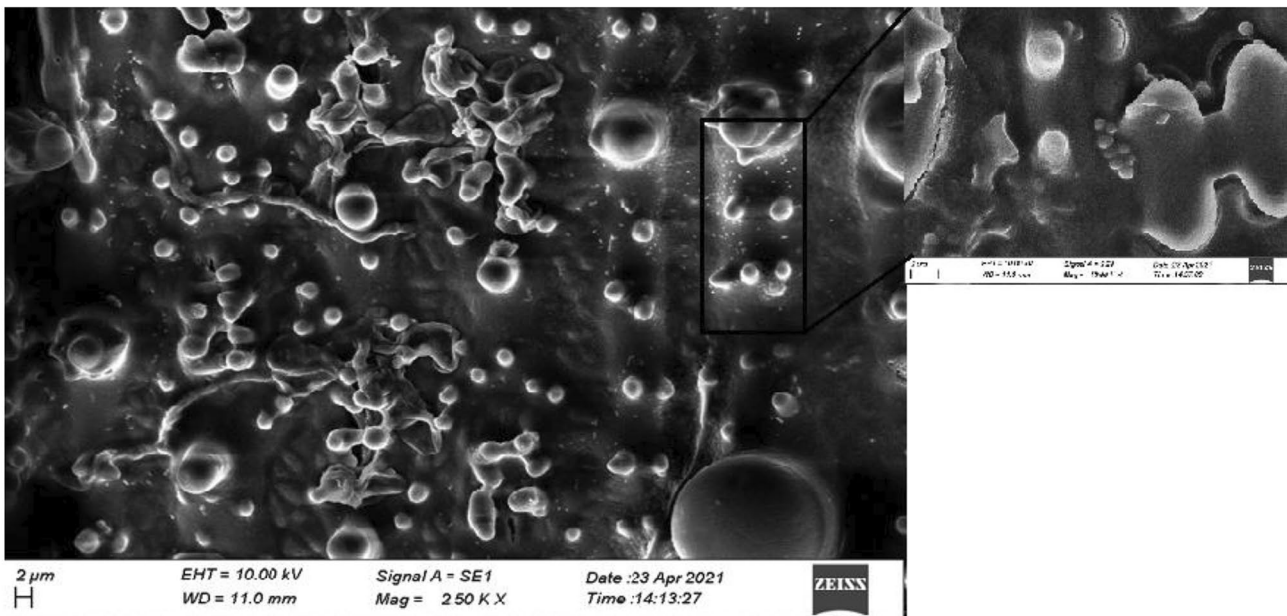
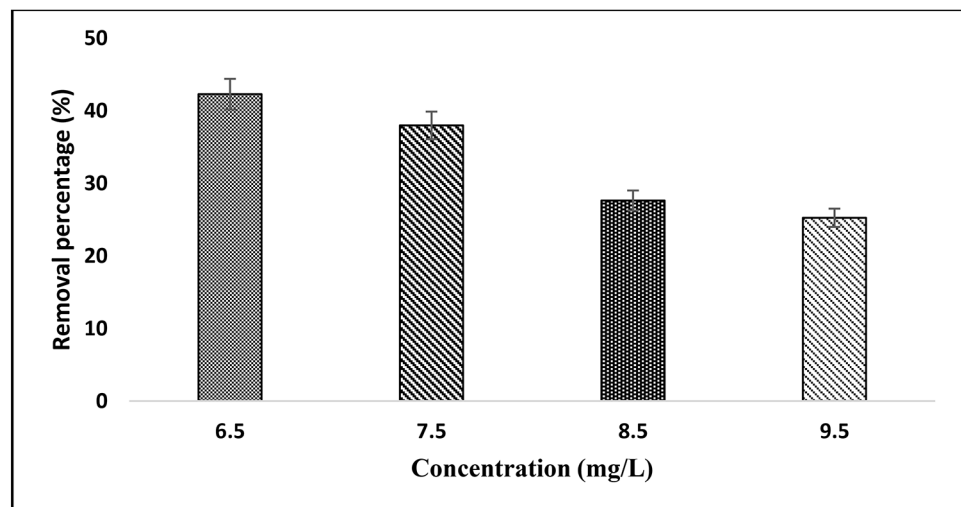


Fig. 5 Scanning Electron Micrographs (SEM) of *S. edaphicus* KCB02A11 on Hay

Meanwhile, ICP-MS analysis in cell-free supernatant exhibited 42.30% of total chromium removal at 6.5 mg/L Cr(VI) which was the highest among other tested concentrations [7.5, 8.5, and 9.5 mg/L Cr(VI)]. Hence, the total chromium removal ability of the *S. edaphicus* KCB02A11 was inversely proportional to the Cr(VI) in the medium (Fig. 4). Considering this total chromium removal pattern of the tested strain, it can be assumed that the total chromium removal potential of the *S. edaphicus* KCB02A11 is curtailed with increasing Cr(VI) concentration.

Biofilm formation and detection

Quantitative biofilm detection results describe that the selected strain can produce “moderately adherent” biofilms according to the standard biofilm detection method proposed by Christensen et al., (1985). This biofilm formation was also screened by scanning electron microscope after they were immobilized on straw. Figure 5 shows the SEM images of the *S. edaphicus* KCB02A11 biofilm on hay (Mag. 2.50 K X) cocci-shaped cells representing *S. edaphicus* KCB02A11 cells (Mag. 10.00 K X) in Tris minimal (modified) medium.

Comparison of removal ability of natural adsorbents, bacterial strain and its biofilms

Comparison of the Cr(VI) removal by selected natural adsorbents; straw and wood husk, *S. edaphicus* KCB02A11 cells in suspension and *S. edaphicus* KCB02A11 biofilm on adsorbents were tested upon exposure to the 8.5 mg L^{-1} Cr(VI), which was the highest concentration of Cr(VI) in which the *S. edaphicus* KCB02A11 biofilms on hay was able to remove completely within 72 h where as *S. edaphicus* KCB02A11 in solution required 96 h to remove the said concentration completely. Straw and wood husk adsorbents reached their maximum removal capacities of 62.25% and 31.39%, respectively, within 72 h. and 48 h. Biofilms of *S. edaphicus* KCB02A11 on wood husk exhibited 45.99% Cr(VI) removal within 96 h. (Fig. 6). However, it was further found that after reaching maximum removal by hay and wood husk adsorbents, a portion of adsorbed Cr(VI) is released back to the medium. Furthermore, the independent samples Kruskal – Wallis test also revealed that, Cr(VI) removal capabilities among adsorbents, bacterial strain and biofilms were significantly different from each other ($p=0.008$, $\alpha=0.05$).

Discussion

Owing to the chemical and physical properties of chromium, such as inert nature, hardness, strength, high-temperature resistance, and corrosion resistance, Cr in its oxidation states of Cr(III) and Cr(VI) are mostly used in metallurgical (67%), followed by refractories (18%), while the rest of the fraction is applied in Cr-induced chemical production, including textile, leather tanning, wood preserving, and pigment production, etc. (Shanker et al. 2005; Saha et al. 2011). Further, it is reported that the above industrial activities are responsible for discharging more than 170,000 tons of waste contaminated with chromium to the ecosystem annually (Kamaludeen et al. 2003).

Humans and other biota like animals, plants, and microorganisms are exposed to these inappropriately discharged chromium through ingestion, inhalation, direct contact with skin, roots, shoots, cell membranes, etc. (Joutey et al. 2015; Coetzee et al. 2020; Kumar et al. 2020; Xu et al. 2021).

Moreover, it is reported that Cr(VI) can cause inhibition of cell growth and cell division, oxidative DNA damage, and morphological changes in the microorganisms (Mishra and Bharagava 2016). Owing to the above hazardous impacts of Cr(VI), local and international authorities responsible for the health and protection of the public and environment have legislated maximum permissible limits for Cr(VI) discharge to the environment.

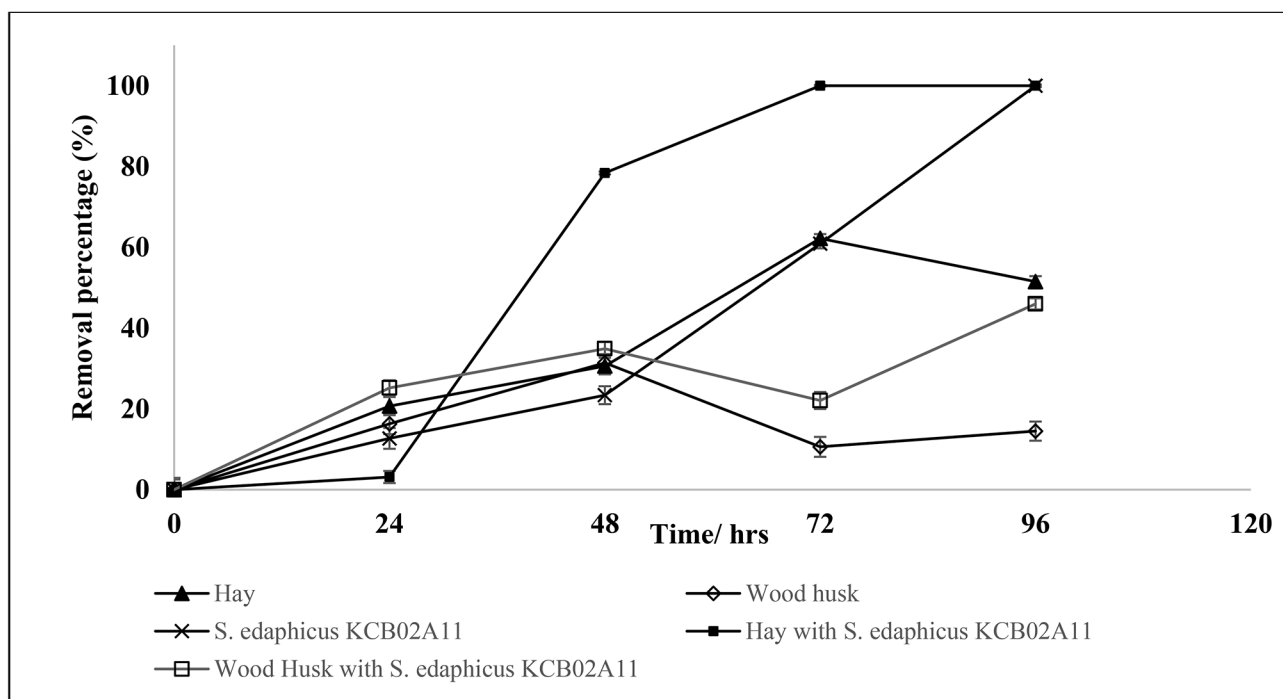


Fig. 6 Cr(VI) removal by *S. edaphicus* KCB02A11 only, adsorbents only and *S. edaphicus* KCB02A11 in biofilms with standard deviation percentile at 95% confidence (Error bars represent standard deviation 0.01–0.197)

The theory behind the bioremediation of Cr(VI) contaminated wastes is to use biological agents that have both Cr(VI) tolerance and removal capabilities with the help of their defence mechanisms such as biotransformation, bio-reduction, and biosorption (Joutey et al. 2015). The usage of Cr(VI) tolerant and removal bacteria for Cr(VI) bioremediation has emerged as one of the most preponderant remediation techniques over the last few decades due to cost-effectiveness and environmental friendliness (Malaviya and Singh 2016; Bhattacharya et al. 2019).

According to dose-response bioassays, *S. edaphicus* KCB02A11 has shown higher tolerance up to 16.0 mg/L of Cr(VI). This bacterium was initially tested for Cr(VI) tolerance in the range of 0.025 mg/L– 0.20 mg/L of Cr(VI) keeping 0.10 mg/L as the middle reference point, which is the maximum regulated Cr(VI) tolerance level legislated by the National Environmental Act of Sri Lanka (National Environmental Act Sri Lanka 2008), followed by gradually increasing the exposure concentration of Cr(VI) up to 16.0 mg/L. Further, it was observed that the isolated strain was viable in all tested Cr(VI) concentrations during the study period of 96 h. The growth response curves of *S. edaphicus* KCB02A11 showed a gradual increase in all tested Cr(VI) concentrations except the highest tested concentration of 16.0 mg/L, which showed a slight growth compared to others. However, none of the Cr concentrations has surpassed the bacterial growth in non-metal added control (Fig. 2). This change of patterns may be due to the toxicity of Cr(VI) towards bacteria.

Previous reports on *Staphylococcus* sp. showed different tolerance limits for Cr(VI), which varied depending on the strain. With providing the high weight of evidence, studies by Zahoor et al., (2009) and Ilias et al., (2011) have reported that wastewater-isolated *S. capitis* and *S. aureus* have higher Cr(VI) tolerance, such as 2800 mg/L and 2000 mg/L, while another comparative study of Pereira et al., (2017) has reported that, *S. saprophyticus* and *S. arlettae* were able to tolerate 300 mg/L of Cr(VI). Furthermore, some strains belonging to the genus *Staphylococcus* were shown to be tolerant up to 500 mg/L (Rajbanshi, 2009) and 25 mg/L of Cr(VI) (Mistry et al. 2010) in their growth medium. Most of the above-tested *Staphylococcus* strains have been isolated from different contaminated sites such as tannery waste, contaminated landfills, and wastewater effluents. Therefore, it can be assumed that the tolerance capabilities of the isolated bacteria may have been influenced by their habitat characteristics. In contrast, it is also believed that some components in conventional growth media could reduce metal toxicity by metal binding, such as glucose and phosphate buffers (Rathnayake et al. 2013). Therefore, the present study used Tris minimal (modified) medium, which

consisted of the least amount of glucose (0.20%) and Tris HCl buffer to optimize the Cr(VI) availability.

Quantitative biofilm detection (Christenson et al., 1985) reveals that the selected strain can produce “moderately adherent” biofilms. This biofilm formation could be another reason for Cr(VI) tolerance of the strain as self-producing extracellular polymeric substances (EPS) confer tolerance to heavy metals (Chien et al. 2013; Yin et al. 2019).

The 50% effective concentration (EC_{50}) estimates (Table 2) of the bacterial strain yielded different values for every 24 h. time interval, decreasing with exposure time up to 96 h. This may be due to the toxicity of Cr(VI) towards bacterial cells. As a common observation, most of the calculated EC_{50} values were below 1 mg/L during the study period. This may be due to effective concentrations strictly depending on the nature of the growth medium (Rathnayake et al. 2013). Therefore, it can be assumed that the lower metal-binding capability of the tris minimal (modified) medium may have influenced EC_{50} values of the tested strain by increasing the Cr(VI) availability. According to the effective concentration analysis, the viability of *S. edaphicus* KCB02A11 can be drastically influenced by Cr(VI) at 96 h. On the contrary, Cr(VI) removal experiments of *S. edaphicus* KCB02A11 exhibit complete Cr(VI) removal reached at 96 h. of exposure. Therefore, it can be assumed that isolated strain may have the ability to remove Cr(VI) even after losing viability, which has been demonstrated by many toxicological studies using different heavy metals, including Cr(VI), and bacterial strains, including *Staphylococcus* sp. (Mukherjee et al. 2018), *Bacillus* sp. (Srinath et al. 2002; Huang et al. 2013; Dadrasnia et al. 2015; Mohapatra et al. 2019), *Kocuria* sp. (Akbarpour Nesheli et al. 2018), *Arthrobacter* sp. (Hlihor et al. 2017), *Variovorax paradoxus*, *Arthrobacter viscosus* (Malkoc et al. 2016), and *Pseudomonas* sp. (Gabr et al. 2008).

Bacterial bioremediation of Cr(VI) can be achieved by biosorption and biotransformation. During the biotransformation, Cr(VI) is reduced to Cr(III). Bacterial strains that can reduce hexavalent chromium are usually known as Chromium Reducing Bacteria (CRB). The Gram-positive CRB is believed to have a significantly high tolerance to higher Cr(VI) concentrations than Gram-negative CRB (Thatoi et al. 2014). Additionally, it has also been reported that chromium tolerance and reduction are independent properties of bacteria (Elangovan et al. 2006; Narayani and Shetty 2013; Thatoi et al. 2014). According to the Cr(VI) removal studies, it was evident that the *S. edaphicus* KCB02A11 could achieve a complete Cr(VI) removal up to the concentration of 8.5 mg/L within the study period of 96 h. followed by a gradual decrease of Cr(VI) removal at higher concentrations (Fig. 3). The results of the Cr(VI) analysis of acid digested cell pellets also indicate the absence of Cr(VI)

inside bacterial cells. Further, ICP-MS analysis of cell-free supernatant revealed a 42.30% of total chromium removal from the 6.5 mg/L Cr(VI) incorporated sample, which was the highest among other tested concentrations (7.5, 8.5, and 9.5 mg/L Cr(VI)). Hence, the total chromium removal ability of the *S. edaphicus* KCB02A11 is inversely proportional to the Cr(VI) in the medium (Fig. 5). Considering this total chromium removal pattern of the tested strain, it can be assumed that the total chromium removal potential of the *S. edaphicus* KCB02A11 is curtailed with increasing Cr(VI) concentrations.

With regard to bacterial Cr resistance, previous studies show that heavy metal tolerance could be accomplished through periplasmic biosorption, intercellular bioaccumulation, and biotransformation (Cervantes and Campos-García 2007; Ramírez-Díaz et al. 2008; Pei et al. 2009; Joutey et al. 2015). Among these mechanisms, biotransformation is considered the best method to tolerate Cr(VI) via reduction to Cr(III), as it extracellularly reacts with functional groups in the bacterial cell wall (Ramírez-Díaz et al. 2008; Joutey et al. 2015). It has also been reported that bacterial EPS contributes to removing Cr(VI) from the medium via negatively charged hydroxyl, carboxyl, phenolic, and sulfhydryl functional groups through reduction, adsorption, and both (Chen et al. 2016; Kang et al. 2017; Tyagi et al. 2020) discloses that bacterial EPS production may also be enhanced with increasing Cr(VI) in the growth medium. No color development was observed in the DPC bioassay conducted with acid-digested biofilm cells, confirming no Cr(VI) inside the cells. Hence, it is assumed that the *S. edaphicus* KCB02A11 biofilm and EPS may have contributed to Cr(VI) reduction and chelation of resulting Cr(III) ions to the cells surface and EPS while the rest of Cr(III) was released back into the medium.

Natural adsorbents such as wood husk, green coconut shells, orange peel, citrus pectin, hazelnut shells, *Terminalia arjuna* nuts, almond shells and wool, etc., are eco-friendly, low-cost materials reported in the literature which can remove Cr(VI) from contaminated sites (Zakaria et al. 2007; Owlad et al. 2009; Kumar and Meikap 2014; Ramirez Losada et al. 2018). Based on the above evidence, Hay and Wood husk were used in the present study. It was revealed that both hay and wood husk provided a matrix for biofilm formation and acted as adsorbents for Cr(VI) removal.

A comparison of the Cr(VI) removal of *S. edaphicus* KCB02A11 in solution and *S. edaphicus* KCB02A11 biofilm on adsorbents were done with respect to the 8.5 mg L⁻¹ Cr(VI), which was the maximum removal concentration of the isolate during the study period of 96 h. The study discovered that *S. edaphicus* KCB02A11 could potentially remove Cr(VI) completely within 96 h. while hay and wood husk adsorbents reached their maximum removal capacities

of 62.25% and 31.39%, respectively, within 72 h. and 48 h. Biofilms of *S. edaphicus* KCB02A11 on wood husk exhibited 45.99% Cr(VI) removal within 96 h., whereas 100% removal on hay within 72 h. (Fig. 4).

However, it was further noticed that after reaching the maximum removal by hay and wood husk adsorbents, a portion of adsorbed Cr(VI) is released back into the medium. This can be minimized by modifying adsorbents either by chemical treatment or physical treatment. Few researchers (Argun et al., 2008; Sciban et al. 2006; Wan Ngah et al., 2008) reported that the efficiency of heavy metal adsorption to natural adsorbents such as rice husk, sawdust, fruit/vegetable waste could be optimized by treating with acids (sulfuric acid, nitric acid, hydrochloric acid, citric acid, tartaric acid), bases (sodium hydroxide, calcium hydroxide, sodium carbonate) and salts (sodium chloride, calcium chloride, zinc chloride). With respect to physical changes, adsorbent capacity can be enhanced by increasing surface area, pore size, pore distribution and adsorbent sites (Gautam et al. 2014). However, this study was mainly focused on bacterial remediation rather than physical or chemical remediation. Therefore, hay and wood husk were mainly used to facilitate biofilm formation, curtailing the operational cost required in consecutive remediation cycles. The present study proved this assumption by providing a high weight of evidence as the *S. edaphicus* KCB02A11 biofilm on hay achieved Cr(VI) remediation in a new batch of contaminants even after 30 days of initial remediation cycle retaining the viability of the aggregated community. The biofilm analysis revealed that *S. edaphicus* KCB02A11 biofilm on hay could be regenerated and reused up to five continuous cycles.

Conclusion

The isolated *Staphylococcus edaphicus* KCB02A11 is a Cr(VI) tolerant bacterial strain having Cr(VI) removal capability with Cr(VI) bioreduction potential. Further, this removal could be optimized with the biofilms formed on the hay, which has excellent potential for its application in the remediation of Cr-contaminated waters.

Author Contribution AMKCBA designed the experiments, conducted the experiments and analyzed the results, contributed to the interpretation of results, and prepared the first draft. IVNR conceptualized the research, secured funding, designed experiments, supervised the study, contributed to the interpretation of results, commented on the first draft, and contributed to the revision of the manuscript. MPD supervised the study and contributed to the revision of the manuscript. MM contributed to the conceptualization of the research, contributed to the interpretation of results, commented on the first draft and contributed to the revision of the manuscript.

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Data Availability All data generated or analysed during this study are included in this published article.

Declarations

Competing interests The authors declare that they have no financial or non-financial interests to disclose

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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