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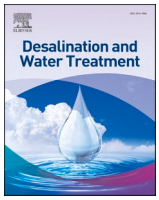

Characterization of Cr(VI)-reducing indigenous bacteria from a long-term tannery waste-contaminated soil

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HIGHLIGHTS

- Four novel Cr (VI) detoxifying bacteria have been characterized.
- The maximum tolerance concentration varies up to 4000 mg/L.
- *Klebsiella quasivariicola* MMKT-15 was best in terms of reduction rate.
- 100 % Cr (VI) reduction was achieved by all the strains at 35 ^{0}C.
- The optimal pH for Cr (VI) detoxification was achieved at 7-8.

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ABSTRACT

This paper investigated the Cr (VI) detoxification potential of 4 novel bacterial strains isolated from a long-term tannery waste-contaminated soil. Molecular techniques were used to identify the bacterial strains using 16 S rDNA gene sequencing. The Cr (VI) detoxification capacity of the bacteria was determined by 1,5-diphenylcarbazide (DPC) methods. The identified bacterial strains were *Bacillus subterraneus* MMKT-10, *Klebsiella quasivariicola* MMKT-15, *Acinetobacter seohaensis* MMKT-19, and *Staphylococcus saprophyticus* MMKT-25. All the strains showed maximum tolerance concentration (MTC) of Cr (VI) up to 4000 mg/L. However, in terms of Cr(VI) reduction rate, *K. quasivariicola* can be considered the most efficient, reducing the two preliminary Cr(VI) concentrations (10 and 20 mg/L) at 15 and 18 h, respectively, while the rest of the strains needed 30 h to reduce the same concentrations from the culture medium. The favorable temperature for Cr(VI) detoxification ranged from 30–40 °C. However, 100 % Cr (VI) reduction was achieved by all the strains at 35 ^{0}C. Interestingly, all the bacterial strains reduced a significant amount of Cr (VI) at 50 ^{0}C, indicative of their thermotolerant nature. The ideal pH for Cr (VI) reduction was 7 for *B. subterraneus* MMKT-10 and *K. quasivariicola* MMKT-15, whereas it was 8 for *Acinetobacter seohaensis* MMKT-19 and *Staphylococcus saprophyticus* MMKT-25. The indigenous bacterial strains isolated in this study could be one of the promising candidates for the detoxification of Cr (VI) contaminated sites.

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1. Introduction

The leather tannery industry (LTI) stands as a significant consumer of freshwater, utilizing it extensively in the production of leather goods. However, this industry also generates a considerable volume of wastewater and sludge laden with harmful organic and inorganic compounds [1]. Notably, the LTI sector in Bangladesh is widely recognized as one of the most environmentally damaging industries, exerting a profound negative impact on the surrounding ecosystem. While LTI operations worldwide employ both chrome and vegetable tanning methods, the predominant use of chrome tanning in Bangladesh contributes to the release of substantial amounts of chromium (Cr) and untreated effluents into the aquatic ecosystem, along with other toxic heavy metals, including lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) [2].

Chromium (Cr) is a hazardous transition metal utilized across various industries, including metal refining, LTI, textile dyeing, and electroplating [3]. Exhibiting multiple oxidation states, from trivalent (Cr(III)) to hexavalent (Cr(VI)), Cr predominantly exists in natural aquatic environments as either Cr(VI) or Cr(III), with varying degrees of stability. Cr(VI) is considered particularly toxic due to its high solubility and rapid permeability into human and animal cells, contrasting with the relatively benign nature of Cr(III), which can be readily absorbed by soil and water [1]. The presence of Cr(VI) and other toxic ions in industrial wastewater poses significant environmental and public health risks, necessitating the implementation of advanced technologies for its removal or remediation [4].

Traditionally, various techniques are commonly employed to eliminate Cr(VI) from wastewater, such as ion exchange, precipitation, ultrafiltration, reverse osmosis and electrodialysis [5]. All these methods experienced a lot of disadvantages like less removal efficacy, the production of huge quantities of chemical sludge, and the high price of chemicals used for Cr(VI) reduction, particularly for the removal of comparatively low concentrations of Cr(VI) in the range of 0–100 μg/L [6]. Hence, there is a pressing need for the development of novel, cost-effective, and environmentally friendly approaches for the removal of Cr(VI) from the contaminated sites [7,8].

Bioremediation emerges as a promising alternative to traditional physicochemical methods, leveraging the capabilities of Cr(VI)-reducing microorganisms for environmental cleanup [6,9]. Bacterial detoxification of Cr(VI) involves the catalysis of Cr(VI) detoxification reactions to Cr(III) by extracellular polymeric substances such as proteins, fats, and carbohydrates. A diverse group of bacteria having minimum inhibitory concentration (MIC) ranging from 22 to 4800 mg/L [10–12] have been reported for their Cr(VI) reduction and removal capacity in a wide range of pH and temperature. Some of the notable bacteria are *Bacillus pumilis, Halomonas sp, Staphylococcus aureus, Corynebacterium hoagie, Arthrobacter sp. WZ2 and Klebsiella pneumoniae* [13–17], to name but a few.

In this study, the Hazaribagh LTI site in Bangladesh, contaminated with long-standing tannery waste, was selected for Cr(VI) pollution remediation. Given the prolonged exposure to Cr(VI) in this area, indigenous bacteria are likely to have developed resistance and Cr(VI) reduction capabilities. However, there is no in-depth study regarding the bioremediation of Cr (VI) pollution from this site. Thus, it is demanded to discover the novel indigenous bacterial strains capable of reducing Cr (VI) to non-toxic Cr(III) to clean up this site effectively.

Thus the specific objectives of this study are to *(I)* isolate and characterize Cr(VI)-reducing bacteria and conduct their molecular characterization through 16S rDNA gene sequencing, *(II)* determine the maximum tolerance concentration (MTC) of Cr(VI) reducing bacterial strains, and (III) optimize the crucial process parameters such as temperature and pH for bacterial Cr(VI)-to-Cr(III) reduction.

2. Experimental details

2.1. Sampling procedures

Tannery waste-contaminated soil was collected from the tannery industrial area of Hazaribagh, Dhaka city, Bangladesh. The samples were collected randomly, making sure that there were about 100 m distance between each sampling site in May 2022. The sampling time was midday. To prevent contamination, samples were collected from a depth of 5 to 10 cm beneath the soil surface. A stainless-steel spatula was used to collect the samples, which were then stored in polythene bags for further laboratory analysis.

2.2. Isolation and enumeration of Cr (VI) tolerant bacteria from tannery waste-contaminated soil and their maximum tolerance concentration (MTC)

To isolate hexavalent chromium [Cr (VI)]-resistant bacteria, 1 g of air-dried soil was placed into a test tube. Subsequently, 9 ml of sterile deionized water was added, and the mixture was vortexed thoroughly. A series of dilutions was generated by combining 1 ml of the suspension with 9 ml of sterile de-ionized water. After that, 0.1 ml of each diluted solution was spread onto nutrient agar plates that contained 100 mg/L of $K_2Cr_2O_7$, which served as the source of Cr (VI), and the spread plate procedures were used. The plates were incubated at 37 °C for 24–72 h. The bacterial colonies were counted using a colony counter at intervals of 24, 48 and 72 h. The viable plate count method was employed to determine the total number of bacteria per gram of soil. A modified broth dilution techniques were followed to assay MTC [7].

2.3. Determination of the reduction potential of Cr (VI) to Cr (III) by the bacterial isolates

The Cr (VI) reduction potentials of the bacteria in this study were assessed using the 1,5-diphenylcarbazide (DPC) method, as proposed by Kabir et al. [7]. Briefly, a measured amount of M9 minimal salt medium and 1 ml of bacterial cell suspensions were inoculated into different centrifuge tubes containing initial Cr (VI) concentrations of 10 and 20 mg/L. Another conical flask containing Cr(VI) and medium served as control. The growth and Cr(VI) reduction were noted at the particular time periods by checking OD at 600 and 540 nm, individually. The Cr (VI) assay procedures were performed using the methods described by Kabir et al. [7]. The total Cr in the culture supernatants was analyzed by using an atomic absorption spectrophotometer (AAS) to cross-check whether all the Cr(VI) was converted to Cr(III) or not.

2.4. Identification of Cr (VI) reducing bacterial isolates and Cr (VI) reduction at various process parameters

Bacterial isolates capable of reducing Cr (VI) were identified using molecular techniques. Two universal bacterial primers, 8 F and 534 R, were employed for this purpose [7,13]. The phylogenetic study of the Cr (VI) reducing bacterial strains was carried out by the maximum likelihood methods using MEGA 7 software. The details procedures of identification techniques have been provided in the supplementary material S1. To examine the impacts of temperature and pH on Cr (VI) detoxification, test tubes were filled with 20 ml of M9 broth containing 20 mg/L Cr (VI). The pH of the broth was adjusted from 0 to 12 using concentrated 1 N HCl or 1 N NaOH. Temperature was varied from 20 °C to 50 °C. Test tubes were then incubated at 37 °C for 24 h. Bacterial growth and Cr (VI) reduction were assessed by measuring optical densities at 600 and 540 nm, respectively.

Table 1
Cr (VI) resistant bacterial load in tannery wasted contaminated soil.

Sample ID	Bacterial load at 24 h (CFU/gm)	Bacterial at 48 h (CFU/gm)	Bacterial load at 72 h (CFU/gm)
S−1	2.88×10^3	3.28×10^3	3.77×10^3
S−2	2.65×10^3	3.01×10^3	3.73×10^3
S−3	2.45×10^3	2.78×10^3	3.23×10^3
S−4	2.36×10^3	2.67×10^3	2.90×10^3
S−5	2.21×10^3	2.53×10^3	2.89×10^3
S−6	2.03×10^3	2.28×10^3	2.58×10^3
S−7	2.97×10^3	3.37×10^3	3.87×10^3
S−8	2.75×10^3	3.27×10^3	3.68×10^3
S−9	2.53×10^3	2.96×10^3	3.47×10^3
S−10	2.40×10^3	2.79×10^3	3.15×10^3
S−11	2.28×10^3	2.61×10^3	2.80×10^3
S−12	2.11×10^3	2.55×10^3	2.73×10^3
Mean	2.47×10^3	2.84×10^3	3.23×10^3
Max	2.97×10^3	3.37×10^3	3.87×10^3
Min	2.03×10^3	2.28×10^3	2.58×10^3

3. Results and discussion

3.1. Isolation, enumeration and identification of Cr (VI) reducing bacterial isolates

The tannery waste-contaminated soil confirmed varying levels of Cr (VI) resistant bacteria at different incubation periods. After incubation for various periods, colony forming units (CFUs) were determined, ranging from 1.80×10^3 to 3.10×10^3 CFU/gm after 24 h, 2.70×10^3 to 3.50×10^3 CFU/gm after 48 h, and 2.90×10^3 to 3.80×10^3 CFU/gm after 72 h, respectively. Additionally, the mean bacterial load present in the soil was 2.75×10^3, 2.84×10^3, and 3.23×10^3 CFU/gm after 24, 48, and 72 h, individually (Table 1). The results indicated that the number of bacteria increased with longer incubation periods, aligning with the findings of previous studies [13,18].

In this study, four bacterial strains capable of removing Cr(VI) under different conditions were successfully isolated. All four strains demonstrated survival at high Cr(VI) concentrations and active removal of Cr (VI). Identification was achieved using universal primers to amplify the 16S rDNA genes. Through 16S rDNA gene sequence analysis, four bacterial strains were identified as *Bacillus subterraneus* MMKT-10, *Klebsiella quasivariicola* MMKT-15, *Acinetobacter seohaensis* MMKT-19, and *Staphylococcus saprophyticus* MMKT-25. BLAST analysis indicated high similarity, with bacterial isolates MMKT-10, MMKT-15, MMKT-19, and MMKT-25 showing 99 % identity to *Bacillus subterraneus*, *Klebsiella quasivariicola*, *Acinetobacter seohaensis*, and *Staphylococcus saprophyticus*, respectively (Table 2). The phylogenetic tree constructed in this study showed a strong association with other Cr (VI) tolerant bacteria (Fig. 1). The Cr (VI) toxicity-minimizing bacteria have been reported in recent years [18,19]. The detoxification and remediation of Cr (VI) polluted sites by *Acinetobacter sp*. has been well documented in different environmental conditions [2,6,13]. Moreover, *Bacillus, Klebsiella, and Staphylococcus* species have also been reported widely for their Cr (VI) tolerance and detoxification characteristics [13,18–20]. The trace metallic contamination in different environmental matrices stimulates the indigenous bacterial communities to be resilient to Cr (VI) and, ultimately, its detoxification to Cr (III) by various specific mechanisms [7, 13,18].

3.2. MTC of Cr (VI) tolerant bacteria at various Cr (VI) concentrations

This study directed to assess the MTC of different bacterial isolates to Cr(VI), as shown in Fig. 2. Out of the total 25 bacterial isolates tested, only four bacterial isolates exhibited higher MTCs to $K_2Cr_2O_7$ as Cr(VI) up to 4000 mg/L at 6–24 h of incubation. On the other hand, other bacterial isolates did not exhibit significant tolerance to $K_2Cr_2O_7$ as Cr (VI) as the concentrations exceeded above 1000 mg/L. Thus, we selected these 4 bacteria for the Cr (VI) reduction studies. The MTCs of Cr(VI) for the bacterial isolates were shown for different times of incubation in Fig. 2. The optical density (OD) of the bacterial isolates were assessed

Table 2
Identified Cr (VI) reducing bacterial strains by molecular techniques.

Bacterial ID	Bacterial name	Identified bacterial strains	Maximum scores	Total scores	E. values	Identity (%)
MMKT−10	*Bacillus subterraneus*	*Bacillus subterraneus* MMKT−10	1249	1068	0.0	100
MMKT−15	*Klebsiella Quasivariicola*	*Klebsiella quasivariicola* MMKT−15	1210	9685	0.0	99.85
MMKT−19	*Acinetobacter seohaensis*	*Acinetobacter seohaensis* MMKT−19	1068	1068	0.0	99.49
MMKT−25	*Staphylococcus saprophyticus*	*Staphylococcus saprophyticus* MMKT−25	1556	8685	0.0	99.87

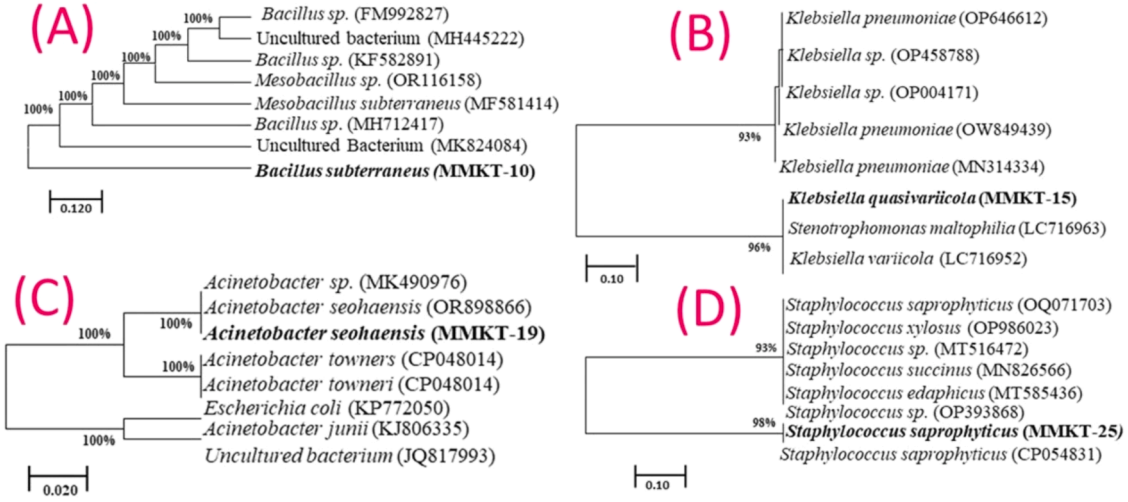

Fig. 1. The phylogenetic trees of the bacterial strains identified in the present study with the other Cr (VI) resistant bacteria constructed by maximum likelihood techniques.

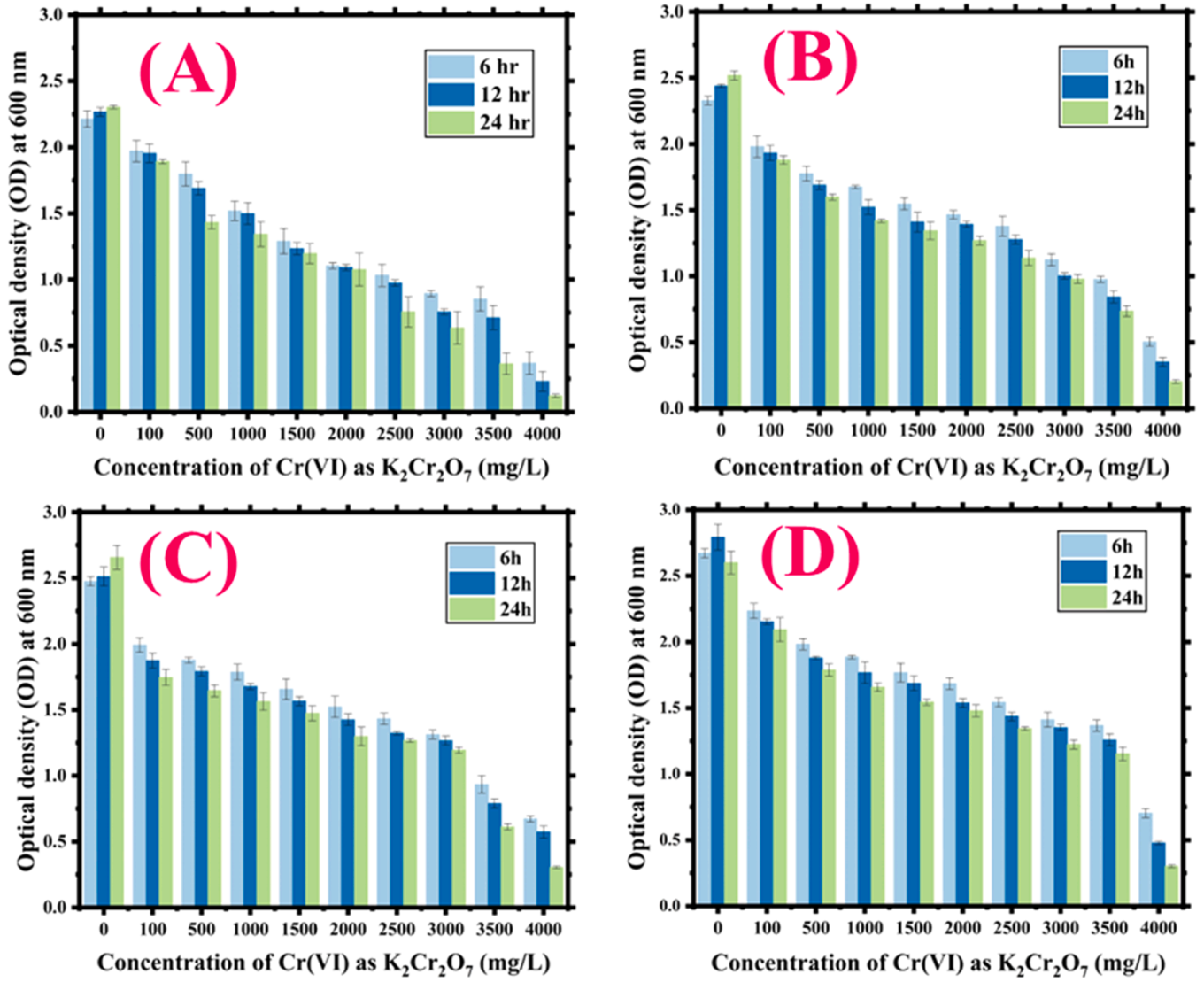

Fig. 2. MTC of **(A)** *B. subterraneu* MMKT-10, **(B)** *K. quasivariicola* MMKT-15, **(C)** *A. seohaensis* MMKT-19 and **(D)** *S. saprophyticus* MMKT-25 at 6, 12 and 24 h of incubation.

after 6, 12, and 24 h of incubation. The OD values diminished with rising concentrations of Cr(VI) [7,13]. All four isolates demonstrated roughly the same tolerance levels at the concentration of 100 mg/L of Cr(VI).

With increasing concentration, the tolerance levels of the four bacterial isolates varied. The highest tested concentration of Cr(VI) was 4000 mg/L, in which MMKT-10 (Fig. 2A) and MMKT-15 (Fig. 2B) showed the highest tolerance at 6 h of incubation with ODs of 0.268 and 0.272 nm, respectively. But, except that, at all concentrations of Cr(VI), MMKT-19 (Fig. 2C) maintained the maximum tolerance level among the bacterial isolates. MMKT-25 (Fig. 2D) kept showing higher tolerance than the other bacterial isolates in all incubation periods until the concentration of Cr(VI) exceeded 3500 mg/L. In 24 h of the incubation period, all four isolates showed MTCs at 4000 mg/L. A sudden drop in the trend of OD values can be seen at the concentration of 4000 mg/L; it may be attributed to the very high level of chromium, which caused antimicrobial toxicity that led to elevated death of the bacteria [21–23].

3.3. Detoxification study of Cr (VI)

The detoxification potentials of Cr (VI) were performed by the highest Cr (VI) tolarent bacteria. M9 broth cultures spiked with 10 mg/L or 20 mg/L Cr(VI) were monitored for bacterial growth and their ability to remediate chromium. Our findings, detailed in Fig. 3, demonstrate the chromium(VI) reduction capabilities of the investigated bacterial isolates. *K. quasivariicola* MMKT-15 achieved a total reduction of both initial Cr(VI) concentrations within 15 and 18 h of incubation (Fig. 3B). *B. subterraneu* MMKT-10 and *Acinetobacter seohaensis* MMKT-19 completely reduced 10 and 20 mg/L Cr(VI) after the incubationof 24 and 30 h, respectively (Figs. 3A and 3C). *S. saprophyticus* MMKT-25 was able to achieve the same reduction after 18 and 24 h, respectively.

This study found a direct correlation between the reduction of Cr(VI) and the proliferation of bacterial isolates, with the process being time-dependent. An additional input to the initial Cr(VI) concentration led to a longer duration for complete reduction [9]. Among the present chromium amount, most of the portion was present in the form of Cr (III)-a reduced version of Cr(VI), demonstrating that all the selected isolates of bacteria achieved extracellular Cr(VI) reduction. This conclusion is further substantiated by the low total chromium levels (Table 3) detected in the culture supernatants. A comparative study of Cr (VI) reduction/removal potential in the existing literature with the present study has been provided in Table 4.

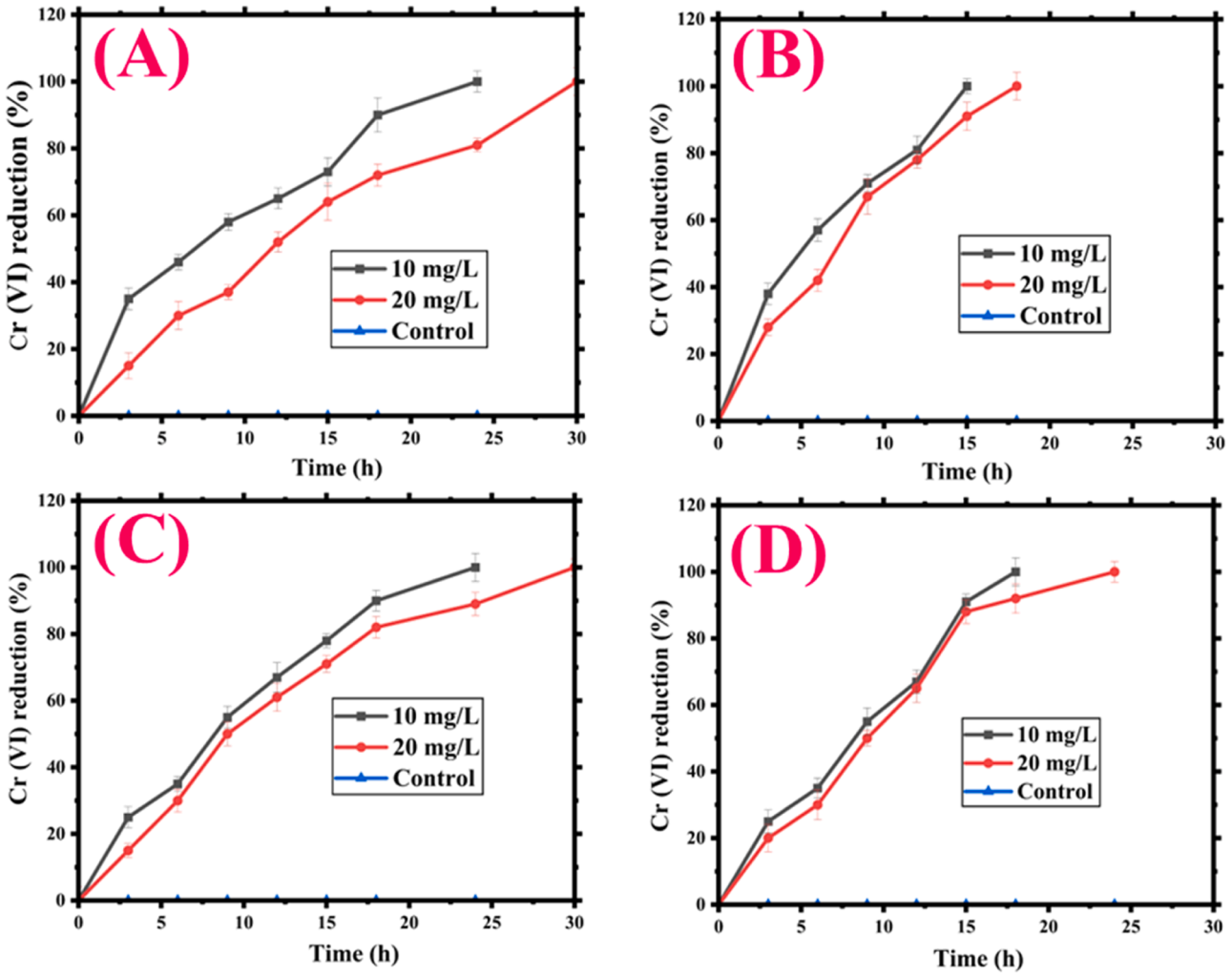

Fig. 3. Detoxification rate of Cr (VI), **(A)** *B. subterraneu* MMKT-10, **(B)** *K. quasivariicola* MMKT-15, **(C)** *A. seohaensis* MMKT-19 and **(D)** *S. saprophyticus* MMKT-25.

Table 3
Total chromium remained in the culture supernatants analyzed by atomic absorption spectrophotometer (AAS).

Bacterial strains	The concentration of total chromium (Cr) in the culture supernatants	
	10 mg/L	20 mg/L
Bacillus subterraneus MMKT−10	9.81 ± 0.38	19.67 ± 0.32
Klebsiella quasivariicola MMKT−15	9.76 ± 0.33	19.88 ± 0.46
Acinetobacter seohaensis MMKT−19	9.95 ± 0.39	19.75 ± 0.41
Staphylococcus saprophyticus MMKT−25	9.84 ± 0.23	19.85 ± 0.51

3.4. Influence of process parameters on Cr (VI) detoxification process

The industrial applications of bacteria are significantly influenced by temperature. It significantly impacts production costs for industrial applications and plays a crucial role in bacterial development. This study evaluated the potential of bacterial isolates to reduce Cr(VI) at a concentration of 20 mg/L across seven temperatures, 20- 50 °C, in an LB broth. Fig. 4 illustrates the temperature's influence on growth and the potential of the bacteria for reducing Cr (VI). The bacterial isolates demonstrated growth and Cr(VI) reduction over a broad temperature range (20–50 °C), out of which optimal performance of the bacteria was observed at 35 °C. For *B. subterraneus* MMKT-10, both growth and Cr(VI) reduction rates rise with an increase in temperature until it reaches 40 °C; beyond that temperature, both parameters sharply decline. (Fig. 4A). The *K. quasivariicola* MMKT-15 showed an increasing trend in growth up to 35 °C, then began to drop at temperatures higher than that; also, the reduction percentage of Cr(IV) remained almost highest at temperatures of 30–40 °C (Fig. 4B). Beyond that, the growth and reduction percentage began to fall like the previous bacterial strains. The growth and reduction percentage of *A. seohaensis* MMKT-19 peaked at 35 °C, then began to decrease (Fig. 4C). The *S. saprophyticus* MMKT-25 demonstrated the highest growth and Cr(IV) reduction at 35 °C. Though the growth began to decline beyond this temperature, the reduction percentage remained almost the highest up to 40 °C (Fig. 4D). In summary, it is observed that 35 °C was the most suitable temperature for the strains for growth and chromium (VI)) reduction, and also 40 °C was the highest tolerable temperature for the bacterial strains.

These results are consistent with earlier studies. Sanjay et al. were able to isolate two bacteria that reduced Cr(VI) [25]. They reported optimal growth along with optimal Cr(VI) reduction rates at temperatures ranging between 35–40 °C. Similarly, Kabir et al. identified optimal temperatures between 35–37 °C for bacterial growth and optimal performance in reducing Cr(VI) [7]. Ramli et al. observed peak growth and Cr(VI) reduction at 35 °C in DM1 [18]. Plestenjak et al. revealed that the bacterium *Amphibacillus sp.* KSUCr3 thrieved and continued optimal performace for reducing Cr(VI) in temperatures up to 40 °C before declining at higher temperatures [24]. Notably, all isolates in the current study revealed Cr(VI) detoxification ability even at 50 °C. Additionally, Camargo et al. observed *P. ambigua* could detoxify Cr (VI) across a broad temperature range between 40–70 °C. Enzymatically

Table 4
Comparison of reported Cr(VI) reducing bacteria with the ones in the present study in terms of Cr(VI) resistant levels, reduction capability and optimum temperatures and pH for growth and Cr(VI) reduction.

SL. No	Bacteria	Initial Conc. (mg/L)	MTC/MIC of Cr (VI) (mg/L)	Optimum temperatures for growth and Cr (VI) reduction		Optimum pH for growth and Cr (VI) reduction		Cr (VI) reduction rate/ percentage	Reduction Time (h)	Ref.
				Growth	Cr(VI) reduction	Growth	Cr(VI) reduction			
1	*Leucobacter chromiireducens* CRB2	100	700	-	30	-	8	100	48	[12]
2	*Bacillus pumilis*	200	-	-	37	-	3	51	24	[17]
3	*Exiguobacterium*	200	-	-	37	-	3	39	24	[17]
4	*Cellulosimicrobium cellulans*	400	-	-	37	-	3	41	24	[17]
5	*Kosakonia cowanii MKPF2*	20	2000	35	40	7	7	100	30	[7]
6	*Klebsiella pneumoniae MKPF5*	20	1800	35	40	7	8	100	30	[7]
7	*Acinetobacter gerneri MKPF7*	20	1800	40	40	7	7	100	24	[7]
8	*Klebsiella variicola MKPF8*	20	1400	35	40	6	7	100	30	[7]
9	*Serratia marcescens MKPF12*	20	2000	35	40	7	7	100	30	[7]
10	*Cellulosimicrobium cellulans*	400	-	-	37	-	7	-	-	[17]
11	*Burkholderia cepacia MCMB−821*	75	-	-	-	-	9	98	36	[19]
12	*Enterobacter sp. HT1*	20	-	-	37	-	7	100	72	[16]
13	*Halomonas sp. M-Cr*	50	-	-	30	-	10	60	24	[15]
14	*Staphylococcus aureus*	20	-	-	37	-	7	100	24	[14]
15	*Pediococcus pentosaceus*	20	-	-	37	-	7	100	24	[14]
16	*Ochrobactrum sp.*	100	-	-	30	-	7	-	-	[11]
17	*Bacillus subterraneus MMKT−10*	20	4,000	40	40	6	5	100	30	Present study
18	*Klebsiella quasivariicola MMKT−15*	20	4,000	35	40	6	7	100	30	Present study
19	*Acinetobacter seohaensis MMKT−19*	20	4,000	35	40	6	7	100	30	Present study
20	*Staphylococcus saprophyticus MMKT−25*	20	4,000	35	40	5	7	100	30	Present study
21	*Corynebacterium hoagii*	10	22	-	-	-	-	69	96	[20]
22	*Arthrobacter sp. WZ2*	100	1000	-	30	-	7	-	-	[21]
23	*Bacillus sp. FY1*	100	1,000		35	-	8		-	[21]
24	*Staphylococcus capitis*	10	4800	37	37	6	7	89	96	[22]
25	*Bacillus sp. JDM−2 −1*	10	2,800	37	37	6	7	83	144	[22]
26	*Ochrobactrum sp. CSCr−3*	100	800	35	35	10	10	-	-	[23]

detoxification of Cr VI) was reported by a novel bacteria, *T. scotoductus,* which remediated Cr (VI) at 60 °C temperature [25–27].

To study the optimal pH for Cr (VI) detoxification, experimental attempts were made at various initial pH values: 2–11, as represented by Fig. 5. Each bacterial strain reduced Cr(VI) across a broad pH ranging from 3–11. The existence of Cr(VI) did not significantly impact the proliferation of the bacteria at different pH levels. *B. subterraneus* MMKT-10 exhibited optimal growth at pH 5.0 and 6.0 (Fig. 5A). The optimal pH for growth was 6.0 for *K. quasivariicola* MMKT-15, and 5.0 to 6.0 for both *A. seohaensis* MMKT-19 and *S. saprophyticus* MMKT-25 (Figs. 5B, 5C, and 5D). The highest Cr(VI) reduction rates for all bacterial isolates were observed within the pH range of 6 to 8.

Bacterial reduction of Cr(VI) is an enzymatic process influenced by pH, which affects enzyme ionization and protein structure, thereby impacting enzyme activity [28]. Notably, *B. subterraneus* MMKT-10 exhibited Cr(VI) reduction at slightly acidic pH 6.0 (99 %), neutral pH 7.0 (100 %), and slightly alkaline pH 8.0 (85 %), despite not showing significant growth at pH 7.0 and 8.0. *P. phragmitetus* LSSE-09 maintained a reduction of Cr(VI) across a broad pH series under aerobic conditions, with optimal reduction at pH 7.0 [29].

Bacteria that are resistant to Cr(VI) maintain a lower pH in their internal cytoplasms than in their environment in alkaline conditions, which helps regulate pH homeostasis [30]. The preference for Cr(VI) detoxification in acidic conditions, due to the positive standard reduction potential of the Cr(VI)/Cr(III) redox couple, may explain the significantly high reduction of Cr(VI) at pH 5 by *S. saprophyticus* MMKT-25 [31–35]. *K. quasivariicola* MMKT-15 showed optimal growth at pH 6 and optimal Cr(VI) detoxification at pH 7, consistent with findings by Kamaludeen et al. that *Klebsiella sp*. optimally reduced Cr(VI) at pH 6 to 7 [36]. An optimal pH range of 7–8 for bacterial Cr(VI) reduction was recorded [37], while Pal et al. identified pH 8 as optimal for *Bacillus* species [38]. For *A. seohaensis* MMKT-19, the finest pH for Cr (VI) remediation was 6–8. Thacker and Madamwar found the most favorable pH limit for Cr(VI) detoxification by *Acinetobacter sp.* to be 6.0–8.0, while pH 7 was recognized as ideal for *Acinetobacter sp.* [39] PCP3 and PD 12 S2 [40–43]. However, Nourbakhsh et al. reported pH 10.0 as optimal for Cr(VI) reduction by *Acinetobacter sp*. AB 1 [39]. The initial pH of the medium significantly affected the reduction of Cr(VI) by *S. saprophyticus* MMKT-25, with the highest reduction observed between pH 6.0 and 8.0. Recent studies reported that the strains of *S. saprophyticus* grew well at pH 8–11, while the optimal growth occurred at pH 9.5 and 8.5 [44–46], demonstrating remarkable tolerance to high alkalinity.

3.5. Feasibility of the present study in terms of selective inoculation, technical design, and operational conditions

The present study offers great possibilities for real-world applications of the novel Cr(VI) reducing bacterial strains to clean up the Cr(VI) contaminated environments. However, in order to apply these bacteria as a bio-stimulant, their metabolic capability, survival proliferation, and pathogenicity profile must be tested in a small-scale soil of Hazarinag

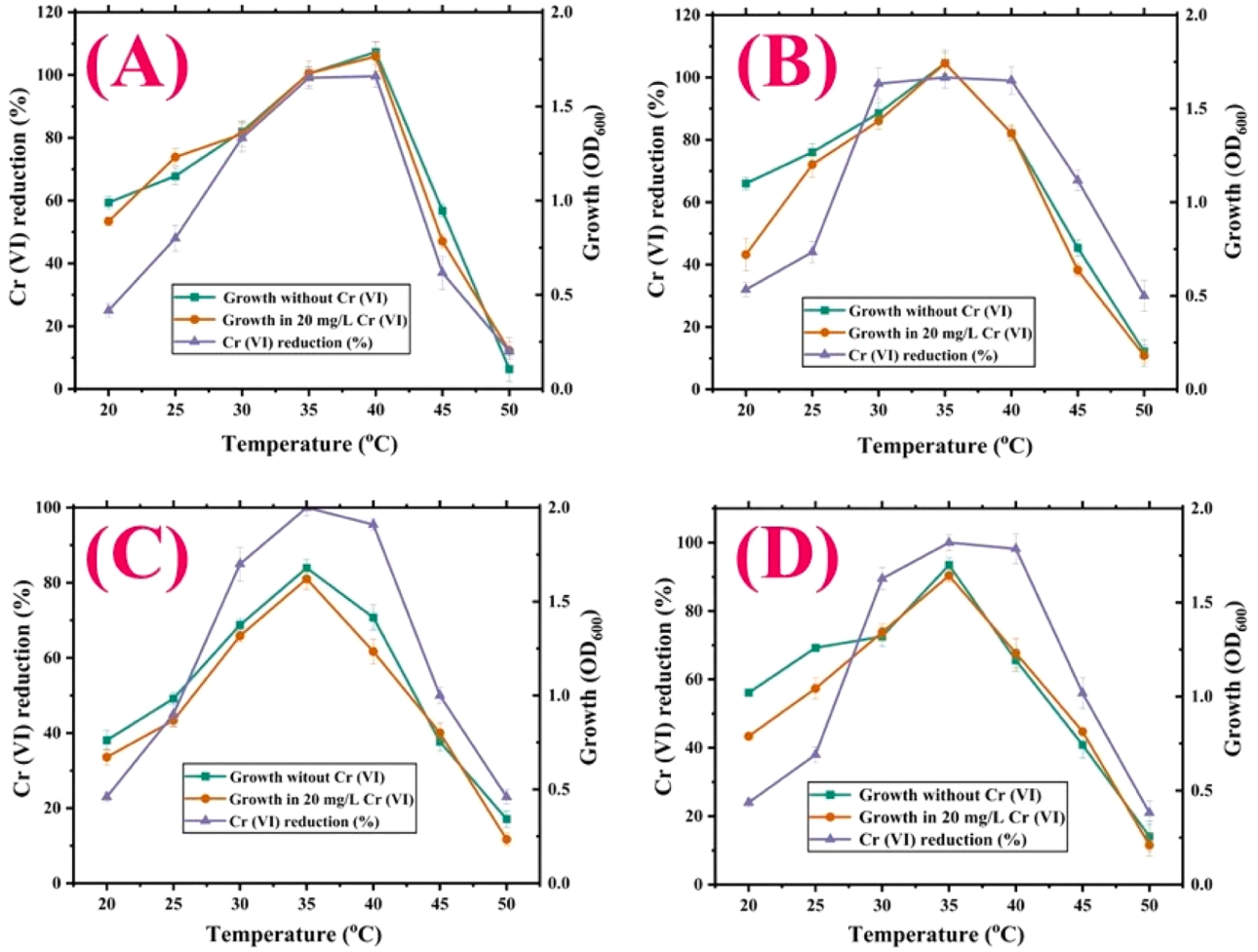

Fig. 4. Detoxification of Cr VI) at varying temperatures, **(A)** *B. subterraneu* MMKT-10, **(B)** *K. quasivariicola* MMKT-15, **(C)** *A. seohaensis* MMKT-19 and **(D)** *S. saprophyticus* MMKT-25.

LTI areas, Bangladesh. Once the best bacterial strains are selected, designing the system for their effective application is crucial. In this case, we shall apply in-situ bioremediation because it is less invasive and often cheaper. To achieve this, it is recommended to develop injection wells for distributing bacteria, bioventing for adding oxygen, and biosparging for injecting nutrients into the Cr (VI) contaminated site. The bacterial strains could be suspended as a liquid media. This Cr(VI) bioremediation process must include methods for monitoring bacterial growth, contaminant levels, oxygen, and nutrient availability to ensure that the bacteria are active and degrading the Cr(VI). As our studied strains could tolerate 30–50 ^{0}C, the temperature should be set up based on these temperatures, and the pH must be within 7–8.

4. Conclusion and outlook

In this study, we characterized 4 novel Cr (VI) detoxifying bacteria from a long-term tannery waste-containing soil, i.e., *Bacillus subterraneus* MMKT-10, *Klebsiella quasivariicola* MMKT-15, *Acinetobacter seohaensis MMKT-19*, and *Staphylococcus saprophyticus MMKT*-25. These bacteria showed significant detoxification potential of hexavalent chromium to trivalent chromium. *K. quasivariicola* MMKT-15 was best, considering its Cr(VI) detoxification rate. The ideal pH and temperature for Cr (VI) detoxification were 7 and 35 ^{0}C, respectively. These strains could be harnessed to create effective bioremediation strategies for toxic Cr VI) contaminated sites in Bangladesh. However, further investigation is needed to scale up this strategy, such as the molecular mechanisms of Cr (VI) detoxification can be studied. The enzymes involved in the reduction process can be characterized. The other process parameters that affect the detoxification strategies can be explored.

CRediT authorship contribution statement

Mohammad Mahbub Kabir: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tania Akter:** Methodology, Investigation, Formal analysis, Data curation, writing – original draft, Writing – review & editing. **Golam Md. Sabur:** Writing – original draft, Validation, Software, Formal analysis, Data curation. **Nazmin Sultana:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Md. Fazlul Karim Mamun:** Writing – review & editing, Validation, Formal analysis, Data curation. **Nasima Kabir:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Md. Didar-ul-Alam:** Writing – review & editing, Visualization, Validation, Data curation. **Mohammed Mafizul Islam:** Visualization, Validation, Investigation, Formal analysis, Data curation. **Farjana Showline Chaity:** Validation, Formal analysis, Data curation. **Leonard Tijing:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Ho Kyong Shon:** Writing – review & editing, Visualization, Validation, Resources, Data curation.

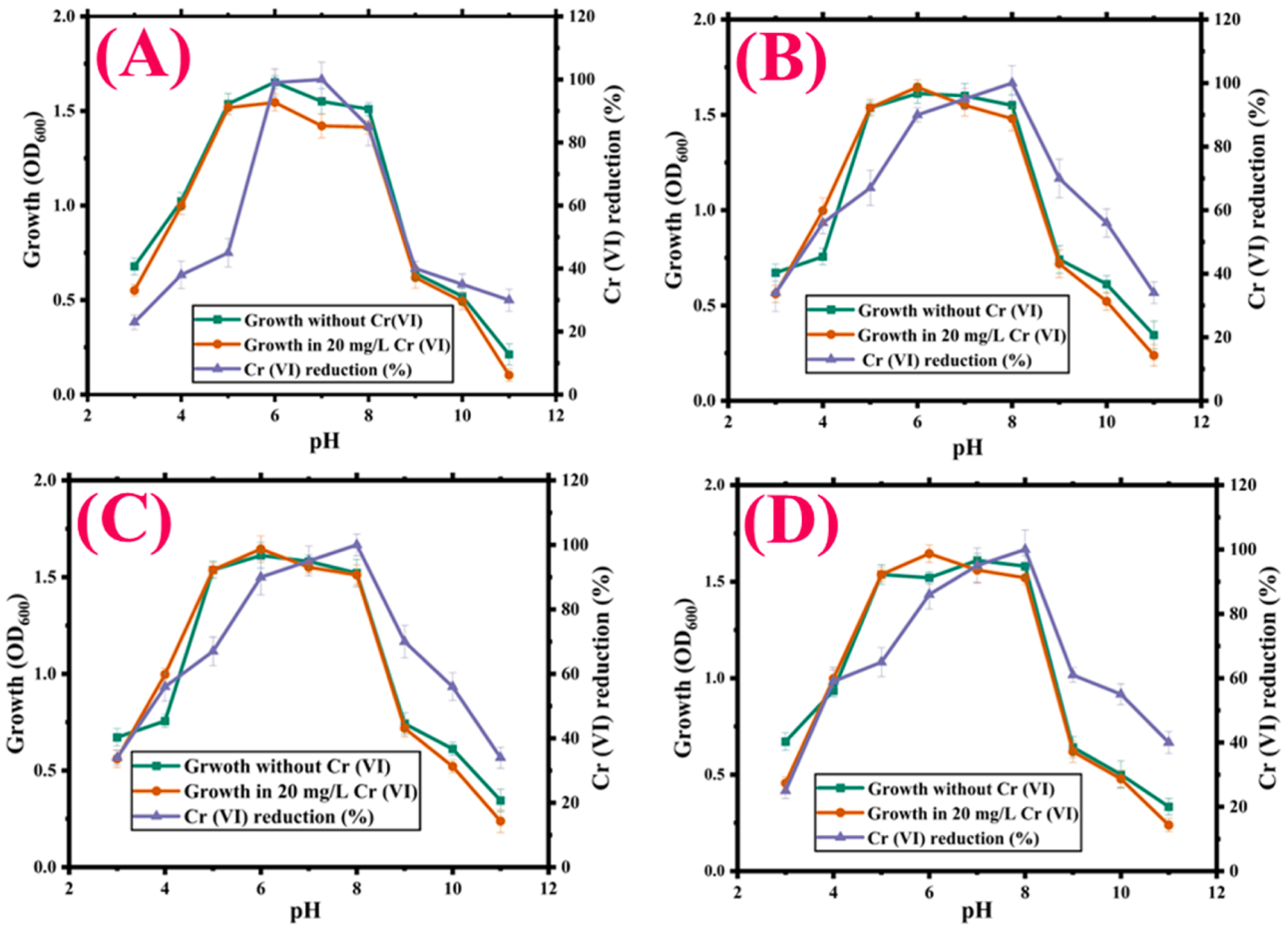

Fig. 5. Cr (VI) detoxification over a wide range of pH, **(A)** *B. subterraneu* MMKT-10, **(B)** *K. quasivariicola* MMKT-15, **(C)** *A. seohaensis* MMKT-19 and **(D)** *S. saprophyticus* MMKT-25.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dwt.2024.100861.

Data Availability

Data will be made available on request.

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