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ORIGINAL ARTICLE

Detoxification of Hexavalent Chromium Using Biofilm-forming *Paenochrobactrum pullorum* Isolated from Tannery Wastewater Effluents

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Abstract

Bioremediation of wastewater using microbes that can detoxify the harmful effects of Cr-VI is considered. Ninety-two isolates were collected from tannery effluents and screened based on their abilities to remediate hexavalent chromium using the LB agar medium supplemented by $K_2Cr_2O_7$ as a source of Cr-VI. Of the total isolates, 44 isolates can grow at 200 ppm of CR-VI, while 7, 6, 21, 11, and 3 isolates showed growth at concentrations of 400, 600, 800, 1000, and 1200 ppm of Cr-VI, respectively. Furthermore, the determination of Cr-VI reduction efficiency for isolates was performed using the 1,5-diphenylcarbazide (DPC) method, and the highest reduction efficiency (70.6%) was achieved by isolate 16 R. This isolate was identified genetically based on the 16S rRNA gene with a higher similarity of 99.83% to *Paenochrobactrum pullorum*, and the genetic tree relationship was constructed. The optimization process was conducted to obtain the optimal reduction conditions, including contact time, pH, temperature, inoculum size, and Cr-VI concentration, using the one-factor-at−a-time (OFAT) method. The highest chromium reduction efficiency increased to 92.5% after optimization for the most potent isolate at pH 9, temperature 30−35 °C, and inoculum size 3−4 ml after 5 days of incubation; biofilm formation was also represented. This study is the first to prove the chromium-reducing characteristic of this strain, along with its ability to form a biofilm, which strongly enhances the use of this strain in reduction and biological treatment processes in wastewater treatment contaminated with Cr-VI.

Keywords: Biofilm remediation, Chromium-detoxification bacteria, Hexavalent chromium, *Paenochrobactrum pullorum*, Wastewater treatment

1. Introduction

All living things depend on water to survive [1]. Globally, water pollution is becoming a major problem that causes illnesses and mortality. Every day, approximately 14,000 people die as a result of water contamination [2,3]. Industrial effluent contamination of water was the most common type of pollution because of the recent rapid industrialization of technology [4]. Several industrial sectors, including metalworking, tanneries, wood conservation, steel manufacture, etc., may introduce toxic metals into the ecosystem through the disposal of wastewater [5−7].

Chromium is a contaminant that has been released by a variety of industrial activities. The environment and human health are both significantly impacted by its toxic effects [8−10]. Only the trivalent and hexavalent forms of chromium, which exist in nine valence levels ranging from −2 to +6 are significant ecologically based on their stability in nature [11].

The physicochemical characteristics and biological reactivity of trivalent and hexavalent chromium

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have different features. While Cr-VI dissolves easily in water, Cr-III is much more difficult to dissolve. In addition, it is known that Cr-VI is very carcinogenic, poisonous, and mutagenic, while Cr-III is a popular dietary supplement and an essential trace element for the metabolism of glucose, lipids, and amino acids [12−14].

To reduce the toxic effect of chromium on ecosystems, we must treat the effluents discharged from tanneries. There are many traditional methods used for reducing chromium, either physically (membrane filtration) or chemically (ion exchange, precipitation). These treatments are useless and very expensive at the small-scale level for treating wastewater with metal concentrations below the range of 100 ppm. Therefore, these technologies must be replaced by much more efficient, inexpensive, and environmentally friendly methods that can successfully reduce small concentrations of chromium [9,15−18].

Numerous studies have demonstrated the use of microbes and their enzyme activities to reduce Cr-VI. It is thought to be one of the most advantageous and useful strategies for converting Cr-VI to the significantly less toxic Cr-III [19], being economical and environmentally pleasant in comparison to conventional techniques [20]. In 1977, *Pseudomonas* from chromate-contaminated sewage sludge was identified as the first bacterial strain. Other strains that have been reported as chromium-reducing strains include *Pseudomonas ambigua, Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus. Subtilis, Bacillus cereus, Achromobacter eurydice, Escherichia coli, Micrococcus roseus, Enterobacter cloacae, Desulfovibrio vulgaris*, and *Desulfovibrio desulfuricans* [21]. A cost-effective and environmentally friendly way to treat wastewater is through the biological reduction of Cr-VI by microorganisms isolated from effluents [22,23].

Biofilm is defined as a microorganism assemblage enclosed in a self-produced matrix of extracellular polymeric substances composed of carbohydrates, water, proteins, and DNA [24]. Observations of biofilm resistance to high-stress conditions have found their way into the bioremediation process [25].

Biofilm-mediated remediation is a cost-effective and environmentally friendly method of cleaning up environmental pollutants [26] because they immobilize, absorb, and degrade a range of environmental contaminants [25]. The role of the biofilm matrix is to protect microbes from severe growth conditions such as desiccation, antimicrobial agents, high acidity, high shear stress, and high concentrations of toxic elements [27,28]. Therefore, the purpose of this research is to isolate, screen, and identify indigenous biofilm-forming and chromium-detoxifying bacteria from industrial wastes and determine the optimal reduction conditions, including contact time, pH, temperature, inoculum size, and Cr-VI concentration, for the most promising strain.

2. Materials and methods

2.1. Chemicals

Analytical-grade chemicals, reagents, and microbiological media were used. Potassium dichromate ($K_2Cr_2O_7$; Merck Millipore Ltd, Bangalore, India) was used to prepare Cr-VI solutions by dissolving in deionized water. 1,5-Diphenylcarbazide has been used for the chromium reduction assay and was prepared by dissolving in 95% acetone [22].

2.2. Sample collection

Samples were collected from the tannery industrial area at the Robbiki Leather City, Cairo, Egypt, and brought to the lab in sterile bottles and kept at 4 °C, where they were used as an inoculum source to get the bacterial isolates that could reduce Cr-VI under aerobic conditions.

2.3. Isolation and selection of the most promising Cr-VI-tolerant bacterial strains

Isolation of the bacterial isolates was done using an enrichment culture technique [29]. Luria−Bertani Broth supplemented with $K_2Cr_2O_7$ at 200 ppm as a source of Cr-VI and 1 ml of wastewater sample that was serially diluted were incubated by shaking at 170 rpm for 12 h at 37 °C. After 12 h, isolate the enriched cultures by spreading them on LB agar plates with 200 ppm of $K_2Cr_2O_7$ and incubating them for 24−36 h at 37 °C. Bacterial colonies with different morphologies were obtained after several streaking and purification. From this preliminary screening, strains that demonstrated good growth with chromium resistance were chosen for further studies [30]. Moreover, to determine the most potent bacteria that can tolerate Cr-VI, the growing bacteria were inoculated in LB agar supplemented with 400, 600, 800, 1000, 1200, and 1400 ppm of $K_2Cr_2O_7$ at 37 °C for 72 h [22,31]. All the obtained isolates were first screened for tolerance against Cr-VI, and the most promising bacteria were selected to be screened for chromium reduction [20,29].

2.4. Assay of chromium reduction efficiency

The Cr-VI reduction was detected by the 1,5-diphenylcarbazide (DPC) method [22] when

measured by spectrophotometry at 540 nm. The presence of Cr-VI is shown by the purple color complex that was formed. Using a standard curve made from a standard solution of $K_2Cr_2O_7$, the following equation was used to determine Cr-VI reduction efficiency of the isolates [32]:

$$\textit{Removal efficiency}\ (\%) = \frac{C_o - C_e}{C_o} \times 100 \quad (1)$$

C_o is the initial concentration of Cr-VI in ppm and C_e is the final concentration of Cr-VI in ppm.

2.5. Identification of the most potent bacterial isolates by 16S rRNA

A promising approach for identifying the phylogenetic relationships between bacteria is 16S rRNA gene sequencing, which is used to identify bacteria from a variety of sources [33]. Online BLAST search tools (http://www.ncbi.nlm.nih.gov/BLAST) were used to compare the acquired sequences to known sequences in the NCBI database, and the similarity and homology of the resulting 16S rRNA sequence were investigated, and the evolutionary history was then inferred [34]. By automatically applying the BioNJ algorithms and Neighbor-Join, pairwise distances obtained with the Tamura-Nei model are applied to a matrix [35].

2.6. Detection of biofilm formation

Supplementing the brain-heart infusion medium (BHI) with 5% sucrose and Congo red is required for the qualitative method to detect bacterial biofilm production. This medium consists of BHI (37 g/L), sucrose (50 g/L), agar (10 g/L), and Congo red stain (0.8 g/L). Congo red was prepared and autoclaved separately from the other medium components to ensure that there were no aggregates and that it was distributed evenly within the medium. This is done because Congo red tends to aggregate in aqueous solutions. After that, it was added to the 55 °C, sucrose-containing, autoclaved brain-heart infusion agar. Incubation occurred aerobically at 37 °C for 24–48 h, and biofilm production was indicated by the appearance of black colonies on CRA plates, while the colonies of biofilm nonproducers remained pink or red [36].

2.7. Optimization of chromium reduction using the one-factor-at–a-time (OFAT) method

The chromium reduction conditions have been optimized in LB media using the OFAT method to establish the optimal Cr-VI reduction efficiency at different contact times of 1, 2, 3, 4, and 5 days; pH values of 5, 7, 8, 9, and 11; temperature degrees of 25, 30, 35, 40, and 45; inoculum sizes of 1, 2, 3, and 4 ml; and Cr-VI concentrations of 100, 200, 300, and 400 ppm of Cr-VI [30,32]. All experiments were performed in triplicate, two times separately, and the data were represented as a mean. Error bars represented each value's standard deviation.

3. Results and discussion

3.1. Isolation and response of bacterial isolates to different Cr-VI concentrations

Samples were obtained from chromium-contaminated wastewater effluents. Wastewater effluents are considered one of the most ideal media for the growth of many bacterial species due to the presence of proteins and chromium [37,38]. A total of 92 isolates were isolated from the tannery industrial area at the Robbiki Leather City, Cairo, Egypt, and using Cr-VI containing the LB medium at a concentration of 200 ppm $K_2Cr_2O_7$, and all isolates were able to show resistance and growth on this concentration. Then, after that, they were further grown on media containing different concentrations of $K_2Cr_2O_7$ between 400 and 1400 ppm (equivalent to 141.6–495.6 ppm of Cr-VI) to determine their growth and tolerance abilities. Table 1 represents the isolates that can grow and tolerate this range of Cr-VI concentrations (400–1400 ppm), which were 48 isolates, and the growth of tolerant strains varied with different chromium concentrations, in which seven isolates (14.58%) are showing strong to moderate growth at 400 ppm, and 6 isolates (12.5%), 21 isolates (43.75%), 11 isolates (22.91%), and 3 isolates (6.25%) are exhibiting strong to moderate growth at concentrations of 600, 800, 1000, and 1200 ppm, respectively. A decrease in bacterial growth was seen along with an increase in the concentration of chromium in the growth media. Similarly, the capabilities of many species of bacteria obtained from tannery effluents to grow at elevated concentrations of Cr-VI were frequently reported by several studies [20,39–42].

3.2. Assay of chromium reduction efficiency

Thirty-seven bacterial isolates were selected based on their ability to survive at several concentrations of Cr-VI to determine their reduction efficiency of Cr-VI at a concentration of 100 ppm. The reduction occurs due to the enzymatic reduction system inside the bacterial cell [43], and the generation of the purple color complex indicated that the Cr-VI is still present.

Table 1. Effect of different concentrations of Cr-VI on the growth of bacterial isolates.

No.	Isolate code	400 ppm	600 ppm	800 ppm	1000 ppm	1200 ppm	1400 ppm
1	196 R	+++	+++	++	++	−	−
2	6 R	++	++	++	−	−	−
3	80 R	+	+	−	−	−	−
4	167 R	+	+	−	−	−	−
5	69 R	+++	+++	++	−	−	−
6	116 R	+++	+++	++	−	−	−
7	65 R	++	+	+	−	−	−
8	66 R	+	+	+	−	−	−
9	130 R	+++	+++	++	+	−	−
10	123 R	+++	++	++	−	−	−
11	49 R	+++	+++	++	−	−	−
12	57 R	+++	+++	−	−	−	−
13	61 R	+++	+++	++	−	−	−
14	84 R	++	++	+	+	−	−
15	159 R	+++	+++	++	+	−	−
16	165 R	+	+	+	+	−	−
17	90 R	++	−	−	−	−	−
18	148 R	+++	++	+	+	−	−
19	93 R	++	−	−	−	−	−
20	99 R	+++	+++	++	−	−	−
21	100 R	++	−	−	−	−	−
22	102 R	+	−	−	−	−	−
23	103 R	+	−	−	−	−	−
24	108 R	+	+	+	−	−	−
25	109 R	++	+	+	−	−	−
26	112 R	+	+	+	−	−	−
27	14 R	+++	+++	++	+	−	−
28	16 R	+++	+++	+++	++	+	−
29	173 R	++	+	+	−	−	−
30	176 R	+++	++	++	−	−	−
31	185 R	+++	++	++	+	−	−
32	188 R	+++	+++	++	+	−	−
33	197 R	++	+	+	−	−	−
34	228 R	+	+	−	−	−	−
35	231 R	+	+	−	−	−	−
36	34 R	+++	+++	++	−	−	−
37	139 R	+++	+++	++	−	−	−
38	140 R	+++	+++	++	−	−	−
39	204 R	+++	+++	++	−	−	−
40	136 R	++	+	+	−	−	−
41	208 R	++	−	−	−	−	−
42	213 R	+++	++	+	+	−	−
43	145 R	+++	+	+	−	−	−
44	32 R	++	−	−	−	−	−
45	216 R	++	+	−	−	−	−
46	134 R	+++	+++	++	−	−	−
47	211 R	+++	+++	++	−	−	−
48	212 R	+++	+++	++	+	−	−

Strong growth (+++), moderate growth (++), weak growth (W), and no growth (−).

The data represented in Table 2 show that the reduction efficiency of the selected isolates in the LB medium supplemented with 100 ppm of Cr-VI is different between the bacterial isolates. The proportion of chromium reduction achieved by 40.5% of the selected isolates was higher than 50%, whereas the lower reduction capacity was achieved by 50.5% of the other bacterial isolates. Out of these bacterial isolates, one unique bacterial strain encoding 16 R exhibited the highest significant growth as well as a good response to chromium at elevated concentrations compared with other isolates. Moreover, the outcomes revealed that the greatest reduction efficiency of Cr-VI was achieved by the isolate 16 R (70.6%); therefore, strain 16 R was selected for further investigations. The greatest reduction capacity of isolate 16 R has appeared when compared with the results obtained by Plestenjak et al. [41], who isolated *P. aeruginosa* 3002 from tannery effluents and were able to reduce about 50% of Cr-VI at a

Table 2. Chromium reduction efficiency of the selected bacterial isolates.

No.	Isolate code	Initial Cr-VI Conc. ppm	Final Cr-VI Conc. ppm	RE (%)
1	159 R	100	51.50	48.5%
2	213 R	100	75.33	24.6%
3	84 R	100	71.17	28.8%
4	165 R	100	66.44	33.5%
5	167 R	100	41.86	58%
6	6 R	100	66.25	33.7%
7	204 R	100	35.24	64.7%
8	49 R	100	72.11	27.8%
9	173 R	100	70.60	29%
10	61 R	100	54.71	45%
11	134 R	100	70.98	29%
12	65 R	100	68.52	31%
13	176 R	100	57.36	42.6%
14	130 R	100	72.68	27%
15	139 R	100	40.72	59%
16	116 R	100	60.58	39%
17	123 R	100	64.93	35%
18	57 R	100	64.74	35%
19	34 R	100	56.61	43%
20	196 R	100	59.82	40%
21	14 R	100	44.50	55%
22	16 R	100	29.37	70.6%
23	185 R	100	67.76	32%
24	212 R	100	65.87	34%
25	148 R	100	42.99	57%
26	194 R	100	44.88	55%
27	66 R	100	42.42	57.5%
28	140 R	100	30.89	69%
29	145 R	100	54.15	45.8%
30	211 R	100	34.10	65.8%
31	99 R	100	45.45	54.5%
32	188 R	100	49.42	50.5%
33	69 R	100	38.83	61%
34	196 R	100	51.27	48.7%
35	130 R	100	62.13	37.8%
36	213 R	100	43.01	56.9%
37	188 R	100	40.09	59.9%

concentration of 100 ppm, while the reduction efficiency lower to 20% at a chromium concentration of 200 ppm. However, Das et al. [43] isolated *Bacillus amyloliquefaciens* CSB 9 strain with the capability to completely reduce 100 ppm of Cr-VI but after a long incubation time (144 h).

3.3. Identification of the most promising isolate by 16S rRNA sequencing

The strain with the highest reduction efficiency, strain 16 R, was selected and identified based on 16S rRNA sequencing, analysis, and alignment of the data to find the relatives most similar to this isolate. Based on nucleotide homology and phylogenetic analysis, isolate 16 R was revealed to be *Paenochrobactrum pullorum* strain 280 (accession no. NR_133808.1) with 99.83% homolog; hence, its identification as *P. pullorum* 16 R. The phylogenetic tree is represented in Fig. 1 [35]. However, the main characteristics of *Paenochrobactrum*-type strains are that their cells are Gram-negative, non-spore-forming, rods of approximately 2.0 mm long. On behalf of biochemical activities, cells are positive in oxidase and catalase tests while negative in urease, nitrate reduction, acid production from sugars, and indole production tests. Finally, this type of strain is growing aerobically [44,45].

3.4. Biofilm formation

In this study, we proved the ability of *P. pullorum* 16 R to form biofilm using the Congo red method. The growth in a biofilm form exhibits enhanced resistance and tolerance to toxic metals. Throughout the cellular metabolic activity, extracellular activity, chemical and physical adsorptions on the cell surface, and photosynthesis, bacterial species transform and remove Cr-VI from wastewater. This is one of the many biological methods used to reduce toxic substances in the environment [38,46]. Our findings showed that the isolates 16 R have the potential to change the color of Congo red brain-heart infusion medium from red to black, which indicates biofilm production (Fig. 2). The presence of extracellular polymeric matrix on the cell surface may assist bacterial cell to resist and overcome the toxic effects of heavy metals like chromium (VI) or adsorb it by chemical adsorption on the cell surface. Wang et al. used an extracellular process to detoxify chromium (VI) from wastewater by *Shewanella loihica* bacteria [47]. The extracellular reduction of Cr-VI is a safer mechanism for its removal, because of the damaging absence < AQ: activity?>of intracellular components and not required for specific transportation channels to transport chromium into the cells [48]. Self-biofilm formation is considered now as an alternative, cheap, and reliable method for wastewater treatment and for sure considered an added value for the nominated strain but still needs further investigation [26,49].

3.5. OFAT results

3.5.1. Effect of different contact times on Cr-VI reduction efficiency

The results of Fig. 3 demonstrated that as the contact time was extended, Cr-VI reduction efficiencies improved as well. The optimum contact time was 5 days with a maximal reduction efficiency of 73.5%. The obtained results are competitive in comparison with data reported by Li et al., who determined the time needed for bioreduction of Cr-VI at a concentration of 20 ppm by *P. aeruginosa*

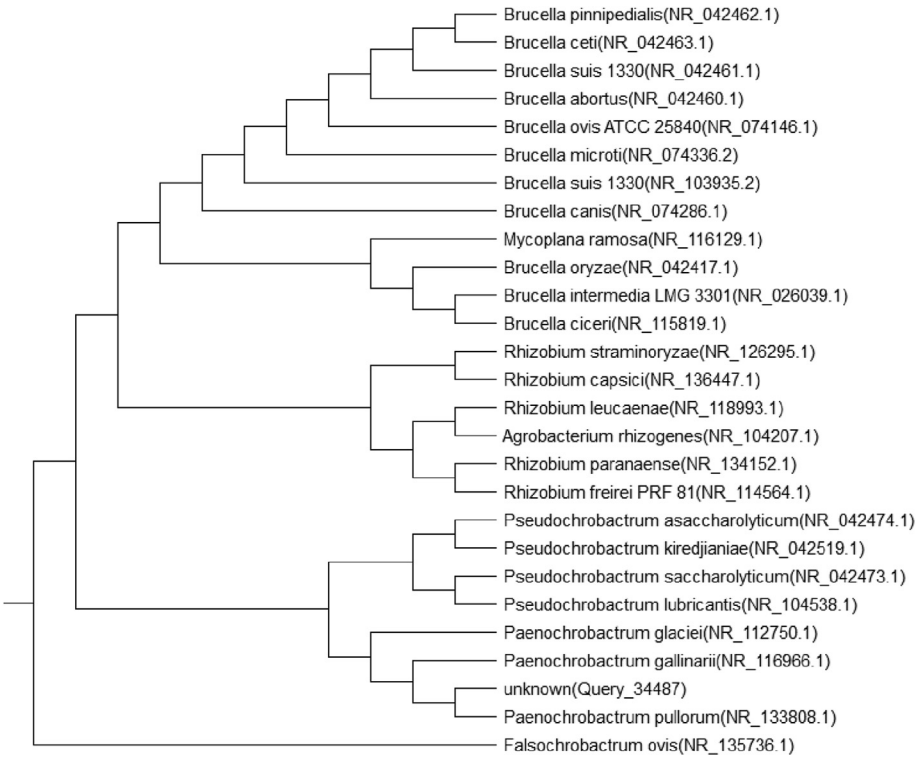

Fig. 1. Phylogenetic tree for Paenochrobactrum pullorum *based on the 16S rRNA gene sequence.*

strain (AB93066) under aerobic conditions was 60 h, and the incubation time was increased to 100 h for bioreduction of 35 ppm of Cr-VI [50]. In addition, the findings of this investigation are in full agreement with the findings observed by Das et al., who isolated *B. amyloliquefaciens*, strain CSB 9, which required 144 h to completely remove 100 ppm of Cr-VI [43]. However, our findings are comparable with the Mishra et al. observations that they isolated *Lactobacillus* strains that can completely detoxify 32 ppm Cr-VI within 6 h [51]. Moreover, some previous studies have reported that, after a specific

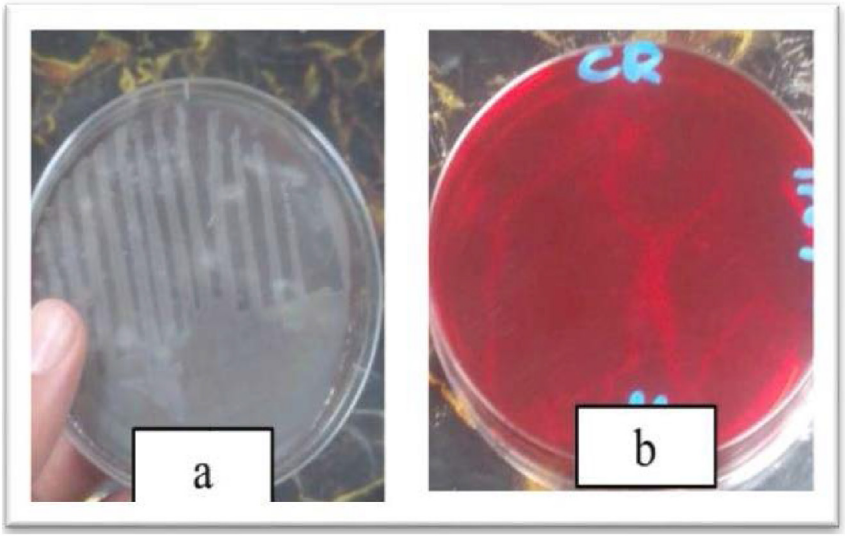

Fig. 2. Biofilm formation by Paenochrobactrum pullorum *16 R: (a) Positive result shows the formation of black color, (b) negative control shows red color of media.*

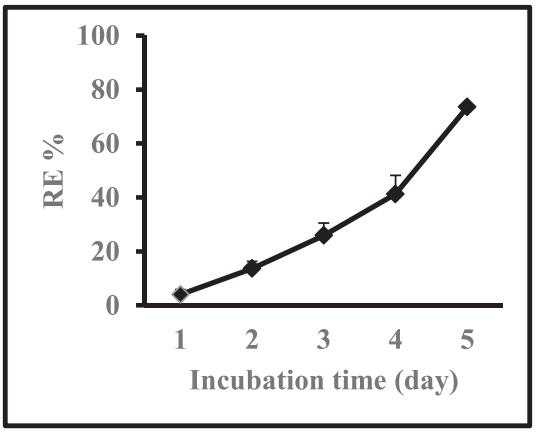

Fig. 3. Effect of different contact times on Cr-VI reduction efficiency.

incubation time, the additional increase in the contact time displayed no effect [52,53].

3.5.2. Effect of different pH values on Cr-VI reduction efficiency

Different pH values were investigated (Fig. 4), which showed that the reduction efficiency of the *P. pullorum* 16 R strain was increased by increasing the pH range from 7 to 9. The highest RE% at pH 7 was 88.3%. An optimal pH level for bacterial Cr-VI reduction has been reported by many other researchers. For instance, a Gram-positive bacterium can reduce Cr-VI at a pH of 9.0, while *P. aeruginosa* and *Bacillus coagulans* can reduce Cr-VI at a pH of 7.0 [54,55]. Ilias and his coauthors reported that the optimum pH value for growth and bioreduction of Cr-VI by bacterial strains IFR-2 and IFR-3 was observed between pH 7.0 and 8.0, whereas the growth and chromium (VI) bioreduction by both strains were considerably lowered at pH values higher than 8.0 [56]. However, the optimal Cr-VI bioreduction observed by Camargo et al. for *Bacillus* isolates were displayed to be related to pH (8.0) [23], while McLean et al. noted that Cr-VI reduction by *P. synxantha* was observed at a wide-ranging pH between 6.0 and 9.0 [30].

The variation in optimal pH values indicates that altering pH is significant for different cultures to achieve the greatest Cr-VI reduction during the bioremediation of chromate [57]. Based on the previous observations, it is observed that the bioreduction is reduced at low pH values, which may be primarily caused by the relationship between bioaccumulation and the quantity of surface-negative charges brought on by the dissociation of functional groups. It has been determined that Cr-VI reduction is enzyme mediated because pH changes have an impact on the rate of enzyme ionization, change protein structure, and subsequently have an impact on the enzyme activity [58–60].

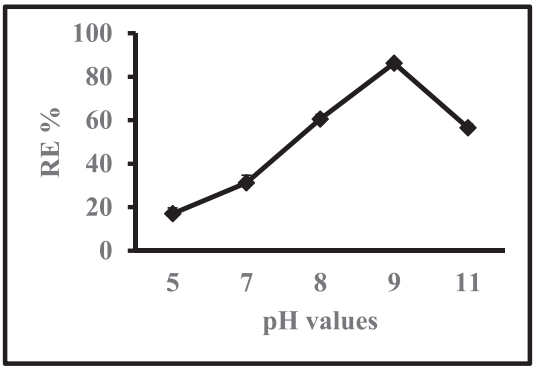

Fig. 4. Effect of different pH values on Cr-VI reduction efficiency.

3.5.3. Effect of different temperatures on Cr-VI reduction efficiency

Temperature has a significant impact on microbial Cr-VI reduction. The bio-detoxification of Cr-VI by *P. pullorum* 16 R isolate was evaluated at five temperature values (25, 30, 35, 40, and 45 °C), and the results are given in Fig. 5. They indicated that the reduction capacity of Cr-VI was reduced effectively at temperatures lower than 30 °C and above 35 °C, while the reduction efficiency was more effective within 30–35 °C and increased up to 92.7%. It agrees with the bioreduction of Cr-VI by *Ochrobactrum intermedium* SDCr-5, for which the optimal temperature was 37 °C [61,62] and also Thacker and Madamwar observed that the maximum bioreduction of Cr-VI by the bacterial isolate DM1 was observed at 35 °C [63]. Tan et al. observed that the *Bacillus* sp. CRB-B1 strain, obtained from sewage sludge, removed 97% of Cr-VI concentration (150 ppm) at 37 °C. Also, Elahi and Rehman noted

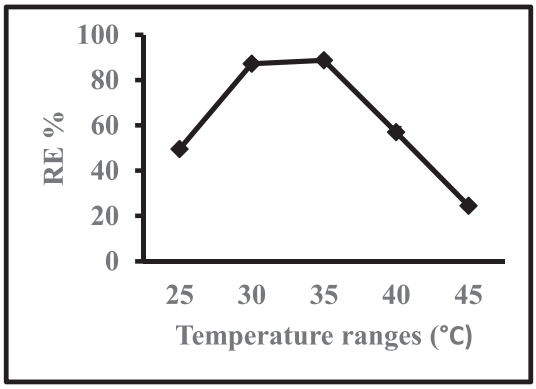

Fig. 5. Effect of different temperature degrees on Cr-VI reduction efficiency.

that the *Staphylococcus sciuri* (A-HS1) strain isolated from tannery effluent reduced 93% of Cr-VI at a concentration of 104 ppm at 37 °C after 6 days of incubation [40,64]. Furthermore, Suzuki et al. isolated *P. ambigua* G-1 with the higher activity to reduce Cr-VI over a wide temperature range from 40 to 70 °C [65].

3.5.4. Effect of different inoculum sizes on Cr-VI reduction efficiency

In this study, the bioreduction of Cr-VI at a concentration of 100 ppm is gradually increased with the increasing of inoculum dose as given in Fig. 6, and the highest Cr-VI reduction was achieved at an inoculum size of between 3 and 4 ml. Many previous studies have reported that one of the significant factors in reducing Cr-VI is initial inoculum size and that there is a linear relationship between it and the inoculum size [22]. Furthermore, as the inoculum dose decreased, the time needed to complete the bioreduction of Cr-VI increased, as reported by Wang et al., who concluded that the bioreduction of Cr-VI wastewater by *Pannonibacter phragmitetus* BB strain was significantly affected by the inoculum size [66].

3.5.5. Effect of different chromium concentrations on Cr-VI reduction efficiency

The effect of chromium concentrations from 100 to 400 ppm was observed during this work. The highest reduction capacity (92.5%) was observed at a concentration of 100 ppm of Cr-VI, as represented in Fig. 7, and the reduction efficiency was decreased with the gradually increased Cr-VI concentration, indicating that the bioreduction potentiality and growth rate were affected with the raising of chromium concentration. Therefore, the data recorded by the previous studies have reported that bacterial growth and RE% decreased when the concentration

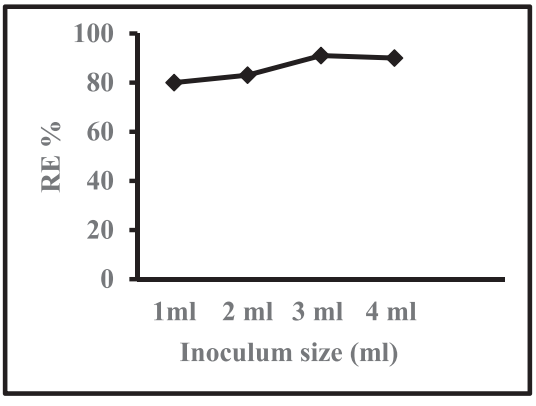

Fig. 6. Effect of different inoculum sizes on Cr-VI reduction efficiency.

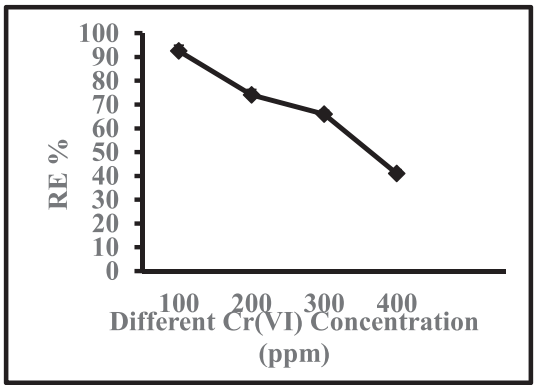

Fig. 7. Effect of different chromium concentrations on Cr-VI reduction efficiency.

of hexavalent chromium in the growth media increased [67]. This is in agreement with Plestenjak et al., who studied the effect of different chromium concentrations on *M. sciuri* and *P. aeruginosa* and reported that at a concentration of 100 ppm of Cr-VI, the bacterial isolates diminished 50% of the Cr-VI after 48 h of incubation while being less active to lower 500 ppm of Cr-VI. This indicated that the last concentration of Cr-VI is highly toxic to bacteria cells [41].

Compared with mobile cells, the presence of biofilm layers on the surface of *P. pullorum* 16 R cells make this strain more stable, easier to reuse, and better at solid–liquid separation and causes less clogging in water treatment systems [62]. This strain may be exploited in the bioremediation of Cr-VI-contaminated environments, particularly wastewater, because its ability to significantly reduce the wide levels of Cr-VI in artificial as well as industrial water samples suggests that this strain has the greatest role in the bioremediation of chromium-polluted environments.

3.6. Conclusions

There is considerable amount of environmental contamination due to the extensive industrial use of chromium. As a result, research for alternate methods to treat environments contaminated with Cr-VI came into existence. To reduce chromium-contaminated environments, microbial remediation, a technology with enormous promise, has gained a lot of attention. So, in this study, the microbial reduction of Cr-VI using a *P. pullorum* 16 R strain isolated from tannery effluent was investigated, and there are no previous reports on the bioreduction of chromium using this strain, so this research may be considered a novel record. Simultaneously, by studying the impact of factors such as contact time,

pH, temperature, inoculum size, and initial chromium content on the development and ability of the isolates to reduce chromium, the best conditions for chromium reduction were found. This study found that the optimum contact time was 5 days; the optimum temperature range was 30−35 °C; and the pH for the strain was found to be 7:9, respectively. Furthermore, the reduction of Cr-VI increased in proportion to inoculum size. After the optimization process, the reduction efficiency increased from 70.6% to 92.5%. This work suggests that the *P. pullorum* 16 R strain can be used to remediate Cr-VI from contaminated industrial effluents. Consequently, it can be concluded that future research can focus on how to utilize improved biotechnology, especially for self-biofilm forming during the removal of Cr-VI, to understand the bioreduction mechanism of chromium bacteria and study the prospects for creating a new strategy for the continuous elimination of chromium (VI) from industrial wastes.

Conflicts of interest

The authors declare no conflicts of interest regarding this manuscript.

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References

[1] Chakkaravarthy DN, Balakrishnan T. Water scarcity-challenging the future. Int J Agric Environ Biotechnol 2019;12: 187−93.
[2] Chaudhry FN, Malik M. Factors affecting water pollution: a review. J Ecosyst Ecography 2017;7:1−3.
[3] Letchinger M. Pollution and water quality, neighbourhood water quality assessment. Project Oceanography 2000;3: 247−9.
[4] Labena A, Abdelhamid AE, Amin AS, Husien S, Hamid L, Safwat G, et al. Removal of methylene blue and Congo red using adsorptive membrane impregnated with dried Ulva fasciata and Sargassum dentifolium. Plants 2021;10:384.
[5] Wuana R, Okieimen F. ISRN Ecology 2011;2011. 2011;10: 402647.
[6] Gunatilake S. Methods of removing heavy metals from industrial wastewater. Methods 2015;1:14.
[7] Faisal M, Hasnain S. Comparative study of Cr (VI) uptake and reduction in industrial effluent by Ochrobactrum intermedium and Brevibacterium sp. Biotechnol Lett 2004;26:1623−8.
[8] Raskin I, Ensley BD. Phytoremediation of toxic metals. New York: John Wiley and Sons; 2000.
[9] Batool A, Din G, Din SU, Jamil J, Hasan F, Khan S, et al. Isolation and screening of chromium resistant bacteria from industrial waste for bioremediation purposes. Braz J Biol 2021;83:125−37.
[10] Bhalerao SA, Sharma AS. Chromium: as an environmental pollutant. International Journal of Current Microbiology and Applied Sciences 2015;4:732−46.
[11] Vijayaraghavan K, Balasubramanian R. Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions. J Environ Manag 2015;160:283−96.
[12] Alvarez AH, Moreno-Sánchez R, Cervantes C. Chromate efflux by means of the ChrA chromate resistance protein from Pseudomonas aeruginosa. J Bacteriol 1999;181:7398−400.
[13] Beszedits S. Chromium removal from industrial wastewaters. Chromium in the Natural and Human Environments 1988;20:231.
[14] Viamajala S, Peyton BM, Sani RK, Apel WA, Petersen JN. Toxic effects of chromium (VI) on anaerobic and aerobic growth of shewanella oneidensis MR-1. Biotechnol Prog 2004;20:87−95.
[15] Pino GH, De Mesquita LMS, Torem ML, Pinto GAS. Biosorption of cadmium by green coconut shell powder. Miner Eng 2006;19:380−7.
[16] Ma Z, Zhu W, Long H, Chai L, Wang Q. Chromate reduction by resting cells of Achromobacter sp. Ch-1 under aerobic conditions. Process Biochem 2007;42:1028−32.
[17] Zayed AM, Terry N. Chromium in the environment: factors affecting biological remediation. Plant Soil 2003;249:139−56.
[18] Dhal B, Thatoi H, Das N, Pandey B. Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: a review. J Hazard Mater 2013;250:272−91.
[19] Thatoi H, Das S, Mishra J, Rath BP, Das N. Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: a review. J Environ Manag 2014;146: 383−99.
[20] Baldiris R, Acosta-Tapia N, Montes A, Hernández J, Vivas-Reyes R. Reduction of hexavalent chromium and detection of chromate reductase (ChrR) in Stenotrophomonas maltophilia. Molecules 2018;23:406.
[21] Romanenko V, Koren'Kov V. Pure culture of bacteria using chromates and bichromates as hydrogen acceptors during development under anaerobic conditions. Mikrobiologiia 1977;46:414−7.
[22] Shreif O, Shehabeldine AM, Abu-Elghait M, Hamed AA, Desouky SE. Statistical optimization of chromium (VI) reduction using response surface methodology (RSM) by newly isolated Stenotrophomonas sp.(a novel strain). Biometals 2022;35:99−114.
[23] Camargo F, Bento F, Okeke B, Frankenberger W. Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate. J Environ Qual 2003;32: 1228−33.
[24] Costerton JW, Cheng K, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, et al. Bacterial biofilms in nature and disease. Annu Rev Microbiol 1987;41:435−64.
[25] Pratt LA, Kolter R. Genetic analyses of bacterial biofilm formation. Curr Opin Microbiol 1999;2:598−603.
[26] Mitra A, Mukhopadhyay S. Biofilm mediated decontamination of pollutants from the environment. Aims Bioengineering 2016;3:44−59.
[27] Davey ME, O'toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 2000;64:847−67.
[28] Mah T-FC, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001;9:34−9.
[29] Nacer A, Boudjema S, Bouhaous M, Boudouaia N, Bengharez Z. Bioremediation of hexavalent chromium by an indigenous bacterium Bacillus cereus S10C1: optimization study using two level full factorial experimental design. Compt Rendus Chem 2021;24(S1):57−70.
[30] McLean JS, Beveridge TJ, Phipps D. Isolation and characterization of a chromium-reducing bacterium from a

chromated copper arsenate-contaminated site. Environ Microbiol 2000;2:611−9.
[31] Zahoor A, Rehman A. Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. J Environ Sci 2009;21:814−20.
[32] Husien S, Labena A, El-Belely E, Mahmoud HM, Hamouda AS. Adsorption studies of hexavalent chromium [Cr (VI)] on micro-scale biomass of Sargassum dentifolium, Seaweed. J Environ Chem Eng 2019;7:103444.
[33] Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ. Multiple sequence alignment with Clustal X. TIBS (Trends Biochem Sci) 1998;23:403−5.
[34] Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 1993;10:512−26.
[35] Holder M, Lewis PO. Phylogeny estimation: traditional and Bayesian approaches. Nat Rev Genet 2003;4:275−84.
[36] Mathur T, Singhal S, Khan S, Upadhyay D, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian J Med Microbiol 2006;24:25−9.
[37] Sivasakthi S, Kanchana D, Usharani G, Saranraj P. Production of plant growth promoting substance by Pseudomonas fluorescens and Bacillus subtilis isolates from paddy rhizosphere soil of Cuddalore District, Tamil Nadu, India. Int J Microbiol Res 2013;4:227−33.
[38] Farag S, Zaki S. Identification of bacterial strains from tannery effluent and reduction of hexavalent chromium. International Journal of Microbiology 2010;31:877.
[39] Princy S, Prabagaran SR. Reduction of Cr (VI) by Bacillus species isolated from tannery effluent contaminated sites of Tamil Nadu, India. Mater Today Proc 2022;48:148−54.
[40] Elahi A, Rehman A. Multiple metal resistance and Cr6+ reduction by bacterium, Staphylococcus sciuri A-HS1, isolated from untreated tannery effluent. J King Saud Univ Sci 2019;31:1005−13.
[41] Plestenjak E, Kraigher B, Leskovec S, Mandic Mulec I, Marković S, Ščančar J, et al. Reduction of hexavalent chromium using bacterial isolates and a microbial community enriched from tannery effluent. Science Report 2022;12:1−11.
[42] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596−9.
[43] Sasmita D, Jigni M, Das S, Sony P, Rao D, Anindita C, et al. Investigation on mechanism of Cr (VI) reduction and removal by Bacillus amyloliquefaciens, a novel chromate tolerant bacterium isolated from chromite mine soil. Chemosphere 2014;96:112−21.
[44] Kämpfer P, Rossello-Mora R, Scholz HC, Welinder-Olsson C, Falsen E, Busse H-J. Description of Pseudochrobactrum gen. nov., with the two species Pseudochrobactrum asaccharolyticum sp. nov. and Pseudochrobactrum saccharolyticum sp. nov. Int J Syst Evol Microbiol 2006;56:1823−9.
[45] Kämpfer P, Huber B, Busse H-J, Scholz HC, Tomaso H, Hotzel H, et al. Ochrobactrum pecoris sp. nov., isolated from farm animals. Int J Syst Evol Microbiol 2011;61:2278−83.
[46] Pradhan D, Sukla LB, Sawyer M, Rahman PK. Recent bioreduction of hexavalent chromium in wastewater treatment: a review. J Ind Eng Chem 2017;55:1−20.
[47] Wang G, Zhang B, Li S, Yang M, Yin C. Simultaneous microbial reduction of vanadium (V) and chromium (VI) by Shewanella loihica PV-4. Bioresour Technol 2017;227:353−8.
[48] Wani PA, Wani JA, Wahid S. Recent advances in the mechanism of detoxification of genotoxic and cytotoxic Cr (VI) by microbes. J Environ Chem Eng 2018;6:3798−807.
[49] Nancharaiah Y, Dodge C, Venugopalan V, Narasimhan S, Francis A. Immobilization of Cr (VI) and its reduction to Cr (III) phosphate by granular biofilms comprising a mixture of microbes. Appl Environ Microbiol 2010;76:2433−8.
[50] Li Y, Wang H, Wu P, Yu L, Rehman S, Wang J, et al. Bioreduction of hexavalent chromium on goethite in the presence of Pseudomonas aeruginosa. Environ Pollut 2020;265: 114765.
[51] Mishra R, Sinha V, Kannan A, Upreti RK. Reduction of chromium-VI by chromium resistant lactobacilli: a prospective bacterium for bioremediation. Toxicol Int 2012;19:25.
[52] Ancona V, Campanale C, Tumolo M, De Paola D, Ardito C, Volpe A, et al. Enhancement of chromium (VI) reduction in microcosms amended with lactate or yeast extract: a laboratory-scale study. Int J Environ Res Publ Health 2020;17:704.
[53] Routray C, Tosh B. Controlled grafting of MMA onto cellulose and cellulose acetate. Cellulose 2012;19:2115−39.
[54] Liu Y-G, Xu W-H, Zeng G-M, Li X, Gao H. Cr (VI) reduction by Bacillus sp. isolated from chromium landfill. Process Biochem 2006;41:1981−6.
[55] Shakoori A, Makhdoom M, Haq R. Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries. Appl Microbiol Biotechnol 2000;53:348−51.
[56] Ilias M, Rafiqullah IM, Debnath BC, Mannan KSB, Hoq M. Isolation and characterization of chromium (VI)-reducing bacteria from tannery effluents. Indian J Microbiol 2011;51: 76−81.
[57] Nguema PF, Luo Z. Aerobic chromium (VI) reduction by chromium-resistant bacteria isolated from activated sludge. Ann Microbiol 2012;62:41−7.
[58] Essahale A, Malki M, Marin I, Moumni M. Hexavalent chromium reduction and accumulation by Acinetobacter AB1 isolated from Fez Tanneries in Morocco. Indian J Microbiol 2012;52:48−53.
[59] Farrell S, Ranallo R. Experiments in biochemistry. A hands-on approach Orlando, FL. 2000.
[60] Arica MY, Bayramoğlu G, Yilmaz M, Bektaş S, Genç Ö. Biosorption of Hg2+, Cd2+, and Zn2+ by Ca-alginate and immobilized wood-rotting fungus Funalia trogii. J Hazard Mater 2004;109:191−9.
[61] Sultan S, Hasnain S. Reduction of toxic hexavalent chromium by Ochrobactrum intermedium strain SDCr-5 stimulated by heavy metals. Bioresour Technol 2007;98:340−4.
[62] Batool R, Hasnain S. Hexavalent chromium reduction by bacteria from tannery effluent. J Microbiol Biotechnol 2012; 22:547−54.
[63] Thacker U, Madamwar D. Reduction of toxic chromium and partial localization of chromium reductase activity in bacterial isolate DM1. World J Microbiol Biotechnol 2005;21: 891−9.
[64] Tan H, Wang C, Zeng G, Luo Y, Li H, Xu H. Bioreduction and biosorption of Cr (VI) by a novel Bacillus sp. CRB-B1 strain. J Hazard Mater 2020;386:121628.
[65] Suzuki T, Miyata N, Horitsu H, Kawai K, Takamizawa K, Tai Y, et al. NAD (P) H-dependent chromium (VI) reductase of Pseudomonas ambigua G-1: a Cr (V) intermediate is formed during the reduction of Cr (VI) to Cr (III). J Bacteriol 1992;174:5340−5.
[66] Wang Y, Peng B, Yang Z, Tang C, Chen Y, Liao Q, et al. Treatment of Cr (VI) contaminated water with Pannonibacter phragmitetus BB. Environ Earth Sci 2014;71:4333−9.
[67] Abo Elazm A, El-Rahim A, Mohamed W, Sabbor A, Moawad H, Sedik M. Bioremediation of Hexavalent Chromium widely discharged in leather tanning effluents. Egypt J Chem 2020;63:2201−12.