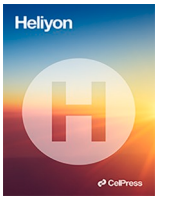
Heliyon 10 (2024) e35650

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Harnessing the chromium reduction potential of *Pseudomonas aeruginosa* JRHM33: A comprehensive study on bioinformatics, phenotype microarray, and CCD-RSM optimization

Jayeshkumar R. Ruparelia [a,b], Hiren K. Patel [b,c,*]

[a] *Shree P.M. Patel Institute of PG Studies & Research in Science, Anand, Gujarat, 388001, India*
[b] *School of Sciences, P P Savani University, Surat, Gujarat, 394125, India*
[c] *School of Agriculture, P P Savani University, Surat, Gujarat, 394125, India*

ARTICLE INFO

Keywords:
Chromium (Cr^{+6})
Optimization
Pseudomonas aeruginosa
CCD-RSM
Laccase
16S rRNA sequencing
Phenotype microarray (PM)

ABSTRACT

Large amounts of wastewater are generated due to overpopulation and industrialization, The bioavailability, toxicity, and permanence of metals make heavy metal contamination a big environmental hazard. In order to maximize chromium (Cr^{+6}) removal efficiency, the current investigation was carried out from industrial wastewater using *Pseudomonas aeruginosa JRHM33*.35 bacterial strains were discovered based on their physical, and biochemical properties and resistance towards chromium (Cr^{+6}) heavy metal. The most significant bacterial strain JRHM33 found the highest-level of 1000 mg/L of chromium (Cr^{+6}) resistance. The bacterial strain JRHM33, which has 99 % similarity to *Pseudomonas aeruginosa*, was found using 16 S rRNA sequencing and is employed in subsequent steps. Sequencing and study of conserved domains indicate that JRHM33 contains the laccase gene and belongs to the multicopper oxidase superfamily, which is known for its ability to reduce metal ions. Analysing phenotype microarray (PM) technology sheds light on *Pseudomonas aeruginosa JRHM33* metabolic profile of microbial cells. Additionally, a series of process parameter optimizations were tried using the central composite design of response surface methodology (CCD-RSM) in an effort to reduce the amount of chromium (Cr^{+6}) in the effluent as much as possible. At 6.8 pH, 90 min of incubation, inoculum size is 3.8 ml, and agitation is 104 rpm, a maximum 71 % Cr^{+6} reduction was attained. The model constructed has an R^2 score of 0.983 indicates a very statistically significant outcome from the analysis of variance. The experimental outcomes and the predicted results were remarkably similar, according to the validation experiment. Studies have revealed that bacterial strains obtained from effluent containing high levels of metals utilize their inherent capability to change harmful heavy metals into less dangerous or harmless forms.

1. Introduction

Industries must be established in areas where the population is growing quickly each day to advance the economy. Because of the constantly expanding industrial sector throughout the world and the continuous discharge of industrial waste into the surrounding environment, mostly in soil, water, and air, numerous dangerous chemicals have accumulated in the environment [1]. This

* Corresponding author. School of Sciences, P P Savani University, Surat, Gujarat, 394125, India.
E-mail address: drhkpatel1@gmail.com (H.K. Patel).

https://doi.org/10.1016/j.heliyon.2024.e35650
Received 21 February 2024; Received in revised form 31 July 2024; Accepted 1 August 2024
Available online 3 August 2024

environmental pollution is at its peak where it can endanger the human population and also other living organisms. Among all the above various types of pollution water pollution draws researcher's attention towards it and rapid growth in industrialization leads to the increase in pollutants in water [2]. Due to the existence of several organic and inorganic contaminants, wastewater discharge can damage the surface and groundwater [3]. The most common inorganic compounds found in polluted sites are metalloids and toxic metals, e.g., arsenic, lead, barium, mercury, cadmium, chromium, nickel, vanadium, and zinc [4–6]. Toxic heavy metal pollution in aqueous streams, which results from untreated metal-containing effluents that are discharged into water bodies, can be toxic to living beings even at low quantities so this needs to be examined and controlled [7]. Chemical or biological processes cannot degrade heavy metals, unlike organic contaminants. As a result, they can only be transformed into less hazardous or adsorbed. Bioremediation is a unique method for cleansing polluted wastewater. Microbial resistance and tolerating to pollutants, especially those containing heavy metals, are very important in bioremediation processes. According to several studies, some bacteria can survive heavy metals by either decomposing them or removing them from the environment into less harmful or completely benign forms, which they then utilize in their metabolic activities to grow [8]. The aim of these study was to use bacteria to remove chromium (Cr^{+6}) ions from a solution in water. We successfully isolated and identified microorganisms that are proficient of condone heavy metals and determination of tolerance capacity of chromium (Cr^{+6}) resistant bacteria [9], Selection of highly potential bacterial strains for bioremediation of chromium (Cr^{+6}). In order to recognize important parameters that affect the process of bacterial isolates removing chromium (Cr^{+6}) from aqueous solution, a central composite design analysis was carried out in the study. To study how various variables, such as inoculum size, pH, incubation time, agitation, and starting chromium concentration (Cr^{+6}), affected heavy metal removal, a Central Composite Design (CCD) was used. An analytical framework for quantifying the impacts of each variable is provided by this experimental design.

The variables of an experiment are simultaneously changing in the statistical design. The primary advantages are the ability to assess the interaction between several factors and to evaluate the effects of various parameters and their relative significance in a specific process [10]. As a result, it employs inexpensive and simple treatments that are widely involved by the public and may often be carried out at the location itself [11].

2. Material and methods

2.1. Sample collection

Sample of water were collected from heavy metal-polluted effluent discharge points of three different electroplating industries and their sludge sample was collected from sludge formation, Makarpura GIDC, Baroda, Gujarat, India. The waste generated from this industry consists of chromium (Cr^{+6}) engaged in the metal plating of car fuel pipes. Sludge and Effluent were collected and transported to the laboratory in sampling vials with screw-cap at a temperature of 4 °C, following the normal protocols, while maintaining aseptic conditions [12].

2.2. Isolation of bacteria

The bacterial strains resistant to heavy metals were obtained using the serial dilution technique on a nutrient agar medium (HiMedia). 1 g of sludge was put off in 10 ml of distilled water Erlenmeyer flasks and distributed by shaking at 200 rpm for 20 min. Every vial was serially diluted up to 10^{-6}. The Spread plate technique was carried out to isolate the organisms from diluted samples. Nutrient agar plates where than topped with 0.1 ml of diluted samples and spread with a glass spreader and incubated for 24 h at 37°C. From the wastewater sample, 10 ml of the contaminated water was directly inoculated into the 100 ml of nutrient broth and it was incubated at 37°C with 150 rpm in shaker for overnight. After 24 h of incubation, The effluent sample was dispersed on the nutrient agar media using 0.1 ml of nutrient broth by a glass spreader and incubated at 37°C for 24 h [13]. The isolates that contain resistance to chromium (Cr^{+6}) was examined in more depth.

2.3. Primary screening of bacteria that are tolerating heavy metal

First, a medium was used to insert each bacterial isolate onto nutrient agar plates individually with a concentration of 100 parts per million of $K_2Cr_2O_4$ in order to screen for heavy metal resistance in the bacteria. Bacterial colony growth was monitored following the preparation of chromium (Cr^{+6}) adjusted nutrient agar, pH was increasing up to 7.5, and plates were incubating for overnight at 37 °C.

2.4. Secondary screening to identify the most effective bacteria that can resistant heavy metal

In order to identify the most effective bacterial strain for heavy metal resistance, a secondary screening process is conducted using two methodologies. Firstly, all bacterial isolates are evaluated for the possibility of heavy metal tolerance. Secondly, the growth of bacteria is observed in the occurrence of chromium (Cr^{+6}), a specific heavy metal.

2.4.1. Determination of heavy metal tolerance test

For the present study on removal of heavy metal chromium (Cr^{+6}), Some concentrations of heavy metals must be tolerable for the bacterial strains. To check the resistance of particular bacterial strains against the chromium (Cr^{+6}) heavy metals the minimal inhibitory concentration [9] test was performed, prepared nutritional agar plates with varying amounts of metals from 100 to 1000

ppm. Metal solutions ($K_2Cr_2O_4$) were prepared in deionized water, filtered to remove impurities, and then put to a nutrient agar substrate. Each of the 0.1 ml bacterial strains was streaked separately on the plates with the various metal concentrations stated above. After that, an incubator was used to incubate all of the plates for 48 h at 37 °C. The presence of heavy metal contaminants in nutritional agar medium was controlled for as a positive control [12].

2.4.2. Growth in the presence of Cr^{+6} metal ion

Testing the bacterial density in the presence of chromium (Cr^{+6}) resulted in adjustments according to Deng and Wang [14] with modifications. Following an overnight incubation at a temperature of 37 °C of a culture in an N-broth medium, chromium (Cr^{+6}) was added to the medium. For each combination, we employed the following concentrations: 0 ppm for control, 25 ppm, 100 ppm, 500 ppm, and 1000 ppm. The cultures were incubated for 48 h at 37 °C in an incubator. A spectrophotometer (Shimadzu UV-1800) was used to quantify the cultures' growth by detecting the change in optical density at a wavelength of 600 nm (OD600).

2.5. Characterization of selected isolate

2.5.1. Biochemical and morphological characterization

Colony morphology was assessed as a function of shape, elevation, margin, surface, and size of most efficient isolates JRHM33, whereas microscopic analysis was conducted by Gram staining. Following Bergey's Manual of Systematic Bacteriology, biochemical analysis was performed on all of the chosen isolates [15].

2.5.2. Metabolic profiling of selected bacterial isolates

Duplicate consumption trends for Biolog carbon substrate inoculations were made using Biolog GP2 MicroPlates (Biolog, Inc., Hayward, CA, USA), which were then incubated at 30–35 °C. Following 12, 24, 36, and 48 h, the decrease produced the optical density at 590 nm. Utilizing a microplate reader and their software (Release version 4.0) to measure the amount of tetrazolium violet in each well [16]. A microplate well was deemed positive if it produced an O.D. 0.59 larger than 0.4 on two or more reading points.

2.5.3. Identification and molecular characterization and phylogenetic evaluation

Using 16 S rDNA sequencing, the molecular identity of JRHM33 was achieved by universal reverse and forward primers (1492 R - 5′ TACGGYTACCTTGTTACGACTT3 and 27 F - 5′ AGAGTTTGATCMTGGCTCAG 3′) using Polymerase Chain Reaction by HiMedia laboratory, Bombay. The NCBI's online BLAST program was used to conduct a similarity search in direction to categorize the isolates. GenBank has received sequenced data for accession numbers. Furthermore, an evolutionary analysis and a neighbor-joining tree were built using NCBI.

2.5.4. Molecular characterization for laccase enzyme

Using bioinformatics, On NCBI, several possible laccase gene sequences were found. Using the In silico rapid PCR software, the gene-specific primer was created [16]. To identify the laccase gene in the bacterial DNA, polymerase chain reaction (PCR) was used. A conserved domain sequence for the laccase enzyme was detected through the use of the "NCBI conserved domain search".

2.6. Screening of significant variables for chromium (Cr^{+6}) removal using Placket- Burman design

It was designed by Plackett and Burman in 1946 [17], variables that may impact the optimal extraction of heavy metal ions, the JRHM33 isolates are employed. The selection of crucial process variables was based on the analysis of existing literature. Subsequently, the percentage of chromium (Cr^{+6}) removal was computed for each assay. Table 1 shows the results of twelve experimental runs, where six variables and five dummy variables were tested. Each variable had two levels. The purpose of these tests was to identify the significant variables. Variables that had a confidence level of 95 % or above were considered to be significant factors in achieving the highest amount of chromium (Cr^{+6}) removal by JRHM33. These variables were then chosen for further optimization at the RSM (CCD) level using the Design-Expert program (version 13.0.1.0).

Table 1
Placket-Burman experimental design was used to remove Cr utilizing independent two-level variables.

Factor	Name	Unit	−1	1
1	pH		4	8
2	Incubation Time	Hours	24	120
3	Initial concentration	PPM	10	100
4	Temperature	°C	25	45
5	Inoculum size	ml	01	05
6	Agitation	RPM	0	150
7	G- Dummy: 1			
8	H- Dummy: 2			
9	I- Dummy: 3			
10	J- Dummy: 4			
11	K- Dummy:5			

2.7. Optimization of selected variables for maximizing chromium (Cr^{+6}) removal by JRHM33 isolates using CCD-RSM

Shake flask studies were carried out using CCD-RSM design wherein four key variables were explored for the optimization of Cr removal. A 30-run experimental design was done, involving four components at five levels (see Table 2). The coded levels were categorized as maximum (+2), high (+1), moderate (0), low (−1), and least (−2). The midpoint is recalculated six times to specifically assess mistakes and the degree of mismatch in the proposed model. The subsequent quadratic equation elucidates the behaviour of the model:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j$$

The predicted response (% of Cr^{+6} eliminated) is denoted by Y. The model constant is represented by β0, while the linear is bi, quadratic is bii, and interaction coefficients is bij. Xi and Xj are the system's independent coded variables [18]. A regression analysis was conducted utilizing the Design-Expert software. With the highest F-value, a p-value below 0.05, and an insignificant Lack of Fit test, the selected model was the one to go. Additionally, a variety of fit statistics measures were employed to assess a proposed model, including the difference between adjusted and projected R^2-values, F-value, R^2-coefficient of determination, and SNR-value of accuracy [19]. Several diagnostic techniques were also used to analysed to the appropriateness of the suggested model. A three-factor interaction study was conducted with counterplots and three-dimensional surface graphs. Lastly, by adjusting the level of various factors, optimization was tried for the highest elimination of heavy metals. Ultimately, experiments were conducted to confirm the model's optimal solution [20].

3. Results and discussion

3.1. Isolation and screening of heavy metal-resistant bacteria

Total number of 40 microorganisms were extracted from industrial sludge. Among 40 isolates, 35 chromium (Cr^{+6}) tolerating strains of bacteria were screened out using the primary screening method [21]. For the purpose of this study, 35 isolates were selected according to their ability to resist chromium (Cr^{+6}). The chromium (Cr^{+6}) concentrations used for this assessment range were from 100 ppm to 1000 ppm. The 35 isolates that were ultimately picked were those with the highest heavy metal tolerance, as determined by a subsequent screening procedure. The initial step was to examine the strains' ability to withstand heavy metals by growing them on a nutrient agar plate that already contained chromium (Cr^{+6}). Among the tested isolates, 23 isolates were tolerating concentrations of 200–300 ppm of Cr, while 10 isolates can handle high level of 600–700 ppm of Chromium (Cr^{+6}) concertation. Interestingly, two isolates, JRHM27 and JRHM33, were able to grow even at a concentration of 1000 ppm of Chromium (Cr^{+6}), and JRHM33 was even able to grow at an extremely high concentration of 10,000 ppm Cr^{+6} (Fig. 1-a).

In the second phase, the most resistant bacteria were selected by incubating selected bacteria with nutrient broth containing chromium (Cr^{+6}) heavy metal. When compared to other isolates, JRHM33 exhibits the highest bacterial growth in the nutritional broth containing Cr^{+6} after 48 h. JRHM33 was therefore chosen for additional research based on growth and tolerance capabilities. Numerous research [22,23] have shown that bacteria isolated from soil contamination and effluent containing different heavy metals exhibit metal resistance. Two bacterial strains were discovered to be resistant to up to 1600 ppm of Cr and Cd [24].

3.2. Biochemical and morphological characterization

Based on heavy metal tolerance capacity and growth in the present Chromium (Cr^{+6}), the gram-negative strain JRHM33 is being studied further as a possible heavy metal removal agent and is shows rod shape bacteria, aerobic, non-capsulated, non-spore-forming, rod-shaped bacterium with unipolar motility and provide a green hue with hints of blue to nutritional agar.

(Fig-1 a, b, and c) and were classified according on their cultural, biochemical, and physical characteristics as determined by Bergey's manual of systematic bacteriology (Table -3). On nutrient agar media, JRHM33 isolate colonies are usually big, smooth, and have an elevated center and flat edge, alternatively, they might be tiny, rough, and convex. According to biochemical analysis, nitrate reduction, citrate, catalase, and oxidase are all positive.

Table 2
Coded and un-coded level of experimental variables of the CCD.

Level	Uncoded level	Coded level			
		Inoculum size (Baumler, Ma et al.)	pH	Incubation Time (Hours)	Agitation (RPM)
alpha	−2	0	2	0	0
Low	−1	1	4	24	1
Mid	0	3	6	72	75
High	+1	5	8	120	150
Alpha	+2	7	10	168	225

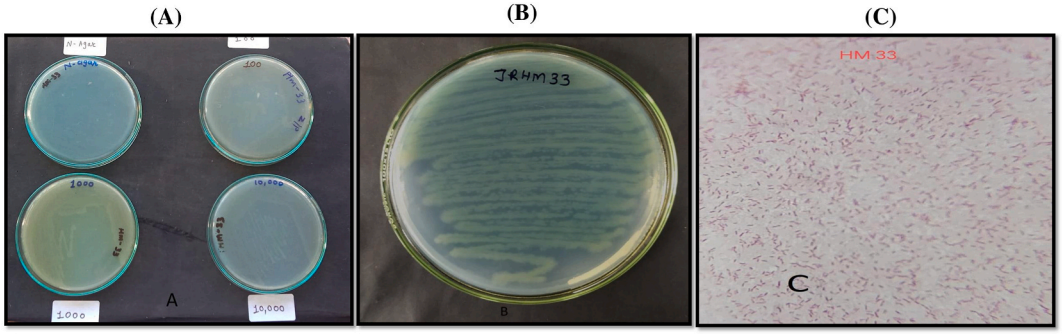


Fig. 1. (A) JRHM33 on nutrient agar plate without heavy metal and with 100 ppm, 500 ppm & 1000 ppm Cr^{+6},(B) heavy metal Growth of pseudomonas aeruginosa JRHM33 on Cr^{+6}-containing nutrient agar and (C) Gram staining of JRHM33.

Table 3
Morphological and biochemical characterization of *Pseudomonas aeruginosa* JRHM33 isolates.

Morphological characteristics	Test	Result
	Colony on Nutrient agar	Smooth, Translucent, low convex, and irregular edge
	Gram's reaction	Gram's Negative
	Motility	Motile
	Cell size & shaped	Small, rod
	Capsule staining	Non-capsulated
	Endospore staining	Non-spore forming
	Pigment	Bluish-green on Nutrient agar
Biochemical characterization	**Test**	**Result**
	Catalase	+
	Oxidase	+
	Citrate	+
	Coagulase	–
	Gelatin hydrolysis	+
	Indole	–
	Methyl red (MR)	–
	Voges Proskauer (VP)	–
	Urease	–
	Nitrate reduction	+
	TSI	Alkali
	Cetrimide	+
	Fructose	+
	Lactose	–
	Mannitol	+
	Sucrose	–
	Xylose	–

3.3. Molecular identification and analysis of potential isolates

The sequence of the 16SrRNA gene was deduced for JRHM33. Using BLAST search, the deduced sequence's very comparable sequence was aligned. Using the 16SrRNA sequencing analysis conducted by NCBI, the sequence was discovered to have a close relationship with the sequence of bacterial strains that were previously documented, with an average identity of 98 %. As a result, the isolates were classified as *Pseudomonas aeruginosa JRHM33*. After being added to the GenBank database, the sequence was assigned the entry number OR105012. The relatedness of the bacterial isolates was shown by the distance tree result (Fig. 2) [25]. Moreover, previous studies have using NCBI's nucleotide BLAST to identify bacteria and fungus through sequencing method, enabling the identification of the organisms at the species level [26–28].

We achieved a 71 % reduction of chromium (Cr^{+6}) with *P. aeruginosa JRHM33* with optimized factor which is quite similar to other researchers, *Mat Arisah* et al.; [29] demonstrated that *P. aeruginosa RW 9* had the capability to eliminate as much as 85 % of 10 mg/L Cr^{+6}. Another study from Shukla, V.Y. et al.; [30] found that the soil's chromium content was reduced by 76 % after treatment. During the 25-day incubation phase, the modified soil was treated with *P. aeruginosa* at a concentration of 4.8×10^9 cells ml^{-1}, along with 1000 μM chromium (Cr^{+6}) kg^{-1} soil. Kafilzadeh, F. et al.; [31] showed that the bacterial isolates *S. marcescens* and *P. aeruginosa* exhibit the ability to thrive and significantly decrease over 95 % of chromium (Cr^{+6}) throughout a broad spectrum of pH values, ranging from 5.0 to 8.0. Other researchers found for zinc and chromium, the percent removal was 65 and 57 % after ten days and it increased after twenty days to 71 and 86 % respectively by Pandian et al.; [32].

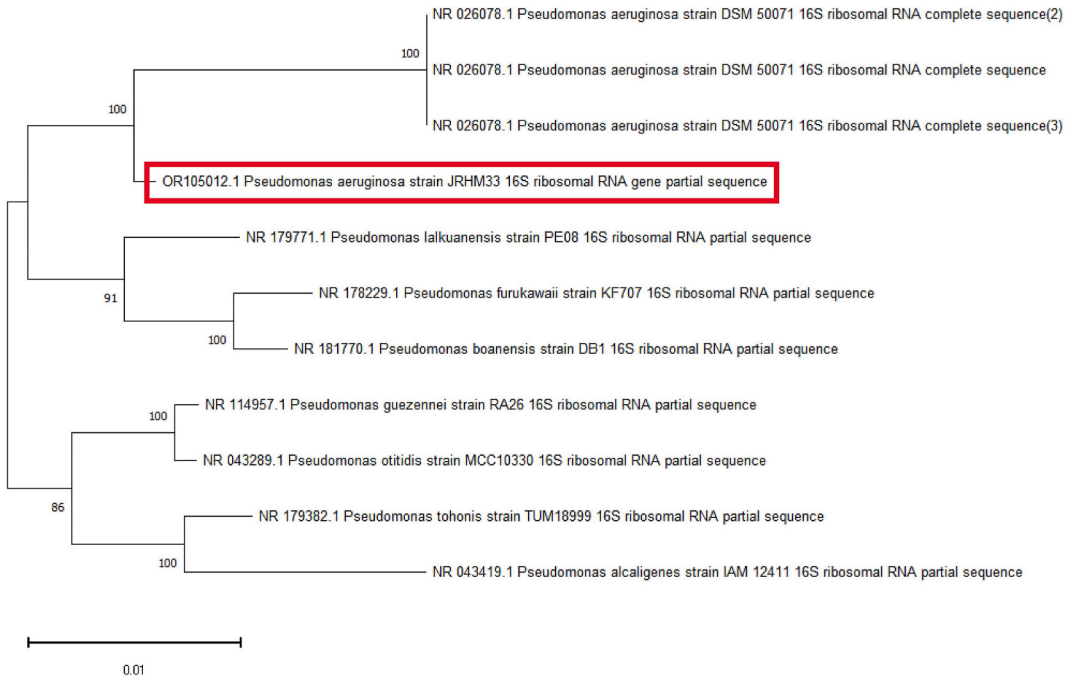


Fig. 2. Phylogenetic analysis of pseudomonas aeruginosa JRHM33.

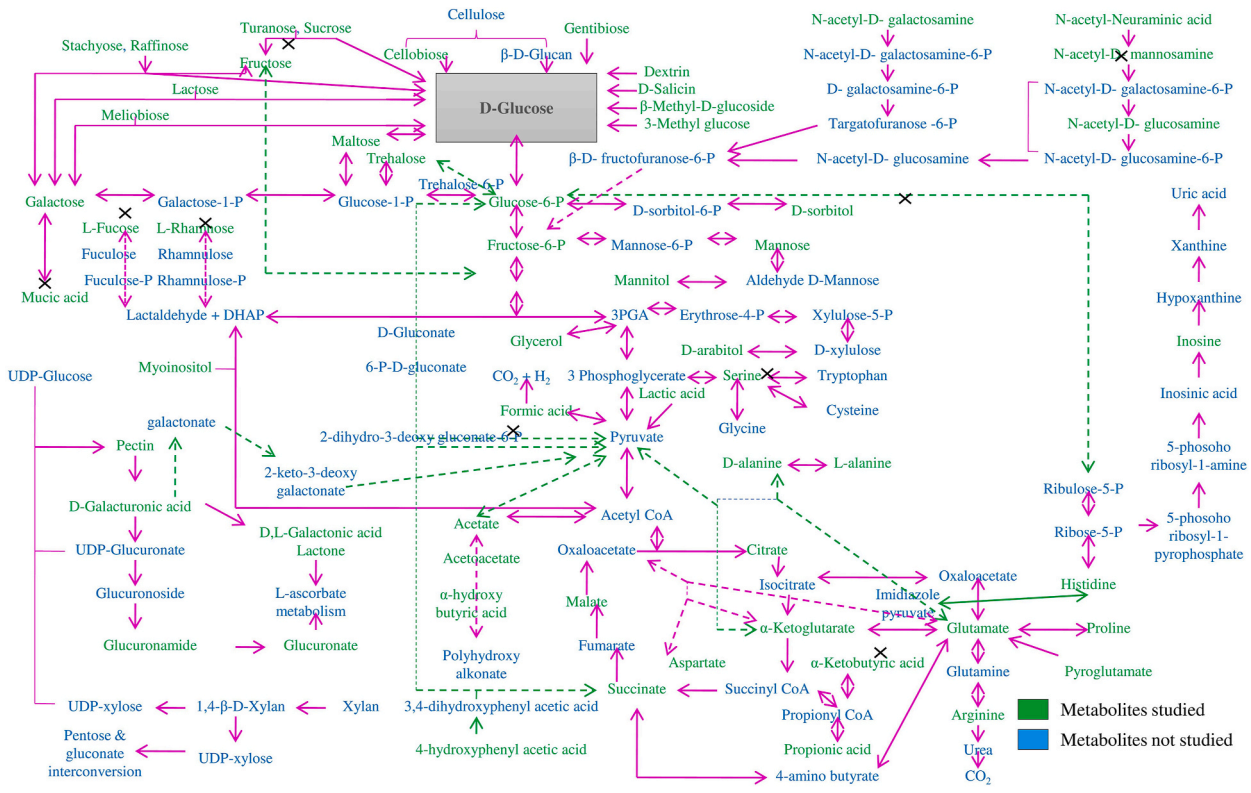


Fig. 3. Carbon source metabolic network of pseudomonas aeruginosa JRHM33.

3.4. Metabolic profiling using Phenotypic microarray (PM) technology

Additionally, we can carefully investigate each isolate's distinct metabolic route through PM technology. Studies on the carbohydrates used by all the different bacteria have shown that these bacterial isolates are able to use basic and complex carbohydrates metabolism. All of the complicated sources, such as α-D-glucose, D-salicin, cellobiose, D-mannitol, Gentibiose, dextrin and D-mannose, etc., were used by *Pseudomonas aeruginosa JRHM33's* metabolic activities (Fig. 3). Glyceraldehyde-3-P was found by Tohsato et al. to serve as a pivotal component that controls the specific metabolic system by its ability to transition between different molecular states [33]. Baumler also looked into the potential uses of carbon, nitrogen, and sulphur by various enterobacteria to identify between species that had been preserved via the PM approach. In addition to revealing variations in the field of systems biology and metabolic networks, the data also revealed a phylogenetic connection between the organism under examination and enterobacteria [34]. The study focuses on comprehending the core metabolic pathway involved in the breakdown of lignocellulose, specifically examining the unique metabolic route in Bacillus sp. NAULH2 responsible for the degradation of banana pseudostems, was made possible by the use of PM technology [16].

3.5. Characterization of laccase gene

Study on the primer sequence revealed that 60.7 °C–64.7 °C is the ideal annealing temperature for Lac CR and Lac CF primers. In order to sequence the laccase gene, a particular band was extracted from the gel and purified using a customized column that came with the Thermo Scientific Genjet kit. Laccase terminal sequences of *Pseudomonas aeruginosa JRHM33's* were 844 bp long. The *Pseudomonas aeruginosa* copper superoxidase genes exhibit a 99 % similarity and are linked to the laccase gene family, was found using the same software's homology search.

To do this, we used the CLUSTAL-W multiple alignment tool to match the amino acid sequences of the *Pseudomonas aeruginosa* laccase genes to those of a previously identified multicopper oxidase family laccase. Three laccase amino acid sequences were analysed using ORF-BLAST to identify the conserved domain of the cupredoxin superfamilies. The laccase domain of the multicopper polyphenol oxidoreductase was identified in the *Pseudomonas aeruginosa* isolate, specifically at amino acid sites 59–788. An analysis of the data reveals the presence of a trinuclear copper binding site inside this domain, belonging to the copper oxidase 4 superfamily (Fig. 4). Bioremediation with laccase enzymes has been identified by numerous researchers as a fruitful method for extracting heavy metal from effluent and soil. Laccase-mediated oxidation transforms the soluble and harmful heavy metal ions into their insoluble and non-toxic state [35]. Additionally, the role of laccase in the bioremediation of chromium involves its enzymatic action in reducing toxic chromium (Cr^{+6}) into less harmful chromium (Cr^{+3}) forms. Other studies have shown that laccase enzymes, produced by fungi like *Ganoderma multipileum*, perform an essential function in chromium biodegradation. Examples like, *Ganoderma multipileum* was used to reduce over 94 % of 100 μg/ml of chromium (Cr^{+6}) into less toxic chromium (Cr^{+3}), showcasing the effectiveness of laccase in chromium reduction [36].

3.6. Screening of significant variables for chromium (Cr^{+6}) removal using Placket- Burman design

The Plackett-Burman design was employed to investigate six process factors and select significant variables for the removal of chromium (Cr^{+6}). There were twelve iterations of the experiment, and five dummy variables were used. You can see the experimental setup and the results (the elimination of chromium Cr^{+6}) in Table 4. The data from the statistical process of the chromium (Cr^{+6}) removal (Table-5) reveal that regarding the chosen process factors There is a positive influence of inoculum size, pH, incubation period, and agitation on the elimination of chromium (Cr^{+6}), and a negative effect of initial concentration. A similar finding to that of [10] is that the percentage of clearance decreases with increasing starting concentrations of metal ions due to the fact that there are more ions competing for the same number of binding sites at the beginning concentration and more binding sites accessible for ion complexation at the beginning concentration. Fig. 5 is a Pareto chart showing the relative importance of the elements; the orange element (dark bar) has a positive effect on chromium (Cr^{+6}) removal, while the blue element (light bar) has a negative effect. The

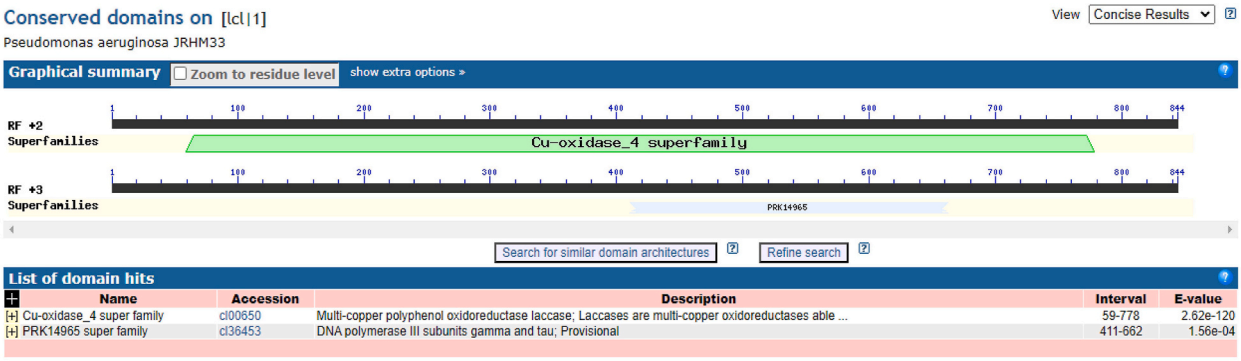


Fig. 4. Conserved domain of laccase gene in pseudomonas aeruginosa JRHM33.

Table −4
coded independent variables for Cr removal by Placket – Burman experimental design.

Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10	Factor 11	Response
	A: pH	B: Incubation time	C: Initial conc	D: Temp	E: Inoculation size	F: Agitation	G: G	H: H	J: J	K: K	L: L	Cr Removal
		Hrs	ppm	C	ml	rpm						%
1	−1	−1	−1	1	−1	1	1	−1	1	1	1	37
2	−1	1	1	−1	1	1	1	−1	−1	−1	1	58
3	1	1	−1	1	1	1	−1	−1	−1	1	−1	72
4	1	−1	1	1	−1	1	1	1	−1	−1	−1	43
5	−1	−1	1	−1	1	1	−1	1	1	1	−1	51
6	−1	1	−1	1	1	−1	1	1	1	−1	−1	59
7	1	1	−1	−1	−1	1	−1	1	1	−1	1	58
8	1	1	1	−1	−1	−1	1	−1	1	1	−1	45
9	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	29
10	1	−1	−1	−1	1	−1	1	1	−1	1	1	58
11	1	−1	1	1	1	−1	−1	−1	1	−1	1	57
12	−1	1	1	1	−1	−1	−1	1	−1	1	1	34

Table 5
Statistical study of the Placket – Burman experimental strategy.

Source	Coefficient estimate	F- value	P-value	
Model		94.81	<0.0001	significant
A-pH	5.42	96.76	<0.0001	
B-Incubation time	4.25	59.56	0.0002	
C-Initial conc	−2.08	14.31	0.0091	
E-Inoculation size	9.08	272.08	<0.0001	
F-Agitation	3.08	31.35	0.0014	

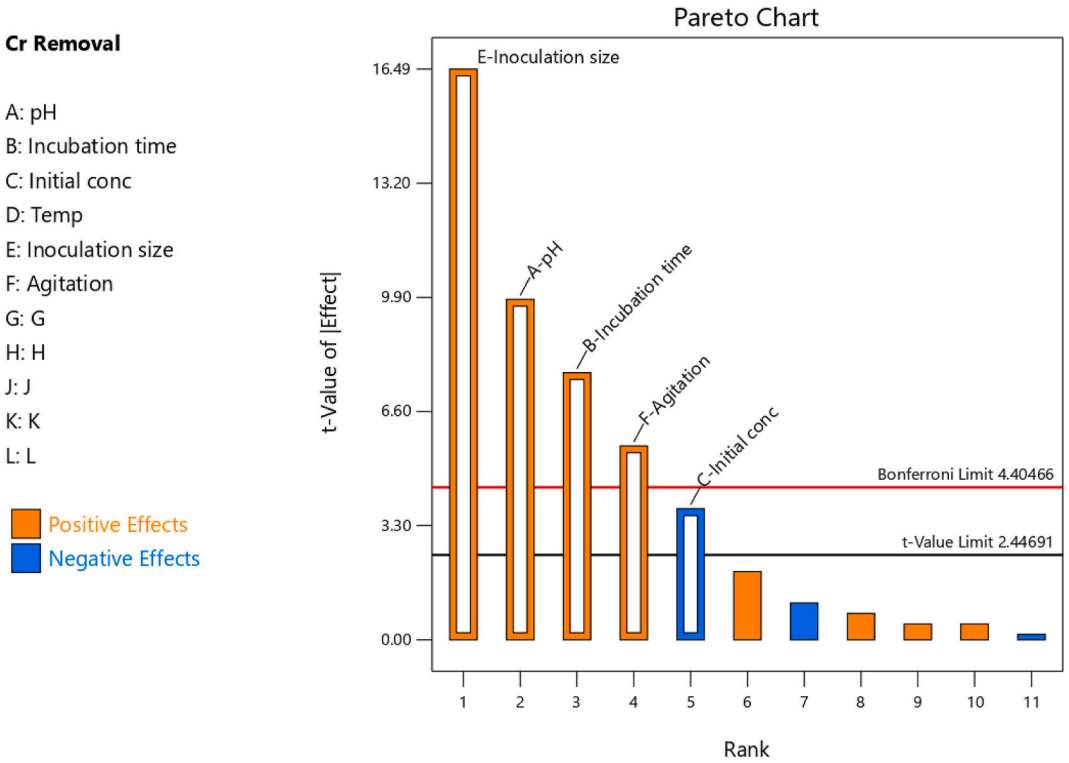


Fig. 5. Pareto chart of 11 factors showing the order of significance.

factors that had a greatly affected on the chromium (Cr^{+6}) removal process, were the size of the inoculum, pH, incubation duration, and agitation. This is clearly demonstrated in Fig. 6, which shows the effect plot as half-normal. Neither the left nor the right side of the linearity line contains any of the four variables. However, due to their tendency to be dispersed along a linear trajectory, the other variables have a lesser effect on the elimination of chromium (Cr^{+6}). There is statistical significance in the model, as shown by the 94.81 model f-value in the ANOVA table. The occurrence of such a large f-value is extremely rare, with just a 0.01 % chance due to noise [37]. The model terms of inoculum size, incubation period, pH and agitation all have p-values less than 0.05, indicating their significance. This outcome is predicated on a 95 % confidence level. The model suggests that the removal of chromium (Cr^{+6}) using JRHM33 isolates is significantly influenced by these four process factors (Table 5).

3.7. A CCD-RSM optimization study for maximizing chromium (Cr^{+6}) removal

The Plackett-Burman design was used to select process factors that have a major effect on chromium (Cr^{+6}) elimination. The CCD-RSM method was then employed to assess the combined impact of these factors and establish the optimal proportion of each variable. A thirty-run experiment was done, with five levels of each of the four variables, using a rotatable design. The experiment included six repetitions at the center point, as shown in Table 6.

The software proposed a statistical-based quadratic model. The F-value was 63.46. The likelihood of a higher F-value resulting from noise is only 0.01 percent. The coefficient of determination, denoted as R^2, assesses how well the model fits the data from the statistics. In this case, the value of R^2 is 0.983, which indicates a strong link between the actual and expected responses. Moreover, the predicted R^2 (0.913) and the adjusted R^2 (0.967) exhibit a difference of less than 0.2 %, indicating a much-required quality for a well-performing model. Furthermore, the suggested paradigm possesses a signal-to-noise ratio of 23.90, indicating its sufficient precision for the

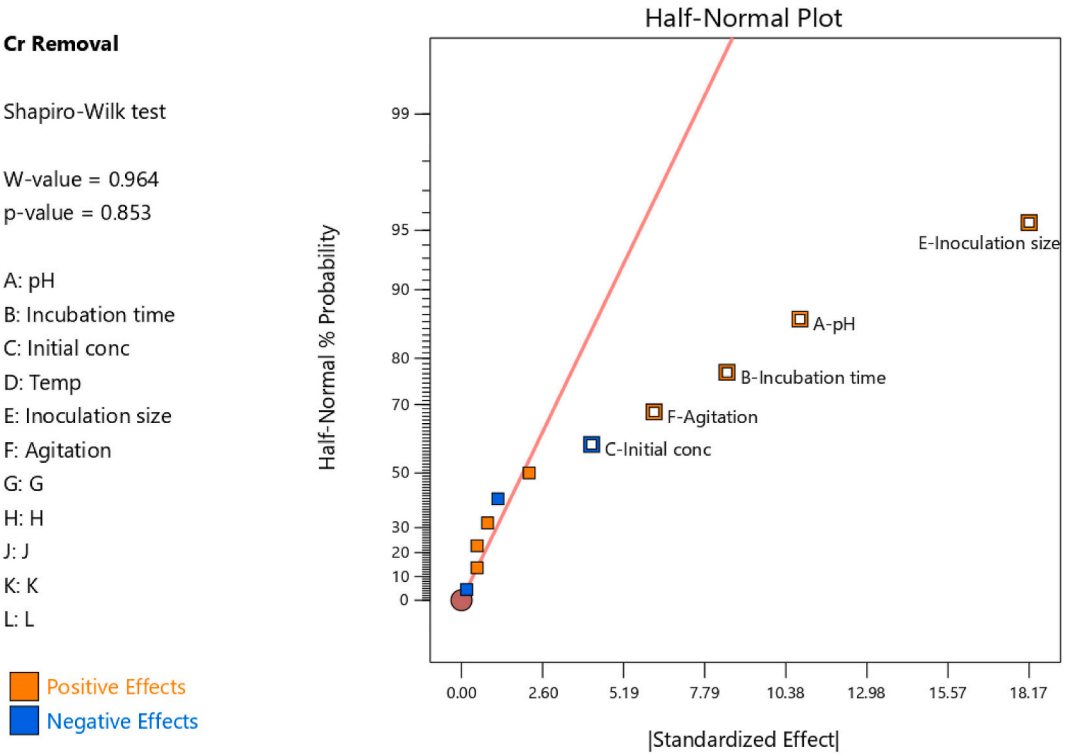


Fig. 6. Half-normal plot displays the important effect of outliers on the Cr removal.

investigation. Based on the F-value of 0.1004, the lack of fit is considered insignificant compared to the pure error. It would be great if the model had a non-significant lack of fit. The appropriateness of the projected quadratic model for improving chromium (Cr^{+6}) removal is confirmed by all of the statistical findings of the fit, as shown in Table 7. Furthermore, the suitability of the proposed paradigm was evaluated by employing the tools for diagnosis and impact within the Design-Expert program. The presence of a linear trend in the normal probability plot suggests that the residuals follow a normal distribution. In order to assess for constant variance, a graph depicting the ratio of residuals to expected replies was analysed. The graph displayed a random distribution of points, suggesting that the variance did not increase as the likely scores were higher. The plot of residuals vs runs showed no noticeable pattern. To determine the necessary power transformation, a box-cox plot was used. At the 95 % confidence level, the graphic reveals that lambda values can range from 0.74 to 1.80, with 1.24 being the optimal number (Fig-7). There was no recommendation for a power transformation because the proposed model has a lambda value that is within the allowed range and is near to the ideal lambda value. All of these results from the diagnostics point to the model being suitable [21].

For each part of this model, we utilized a second-order polynomial equation to get the job done and forecast the response based on the numbers we provided. When we compare the factor coefficients, we can see how much of an impact each component has:

$$Cr\ removal = +67.83 + 7.25A - 4.83B - 3.92C + 4.58D + 2.88AB - 1.0000\,AC + 0.7500AD + 0.1250BC + 0.6250BD + 3.75\,CD - 9.54\,A^2 - 11.29\,B^2 - 7.29\,C^2 - 6.79\,D^2$$

A two-dimensional contour plot and a three-dimensional surface plot were created to analyse the effects of interactions on the chosen control parameters. These plots depict the response variable versus two other numeric components, while keeping the remaining parameters fixed at Fig. 8 shows the average values. The findings indicate whose effect of curvature had the seen, demonstrating the interaction impact of the chosen independent factors in achieving the maximum level of chromium (Cr^{+6}) heavy metal removal. The peak point of chromium (Cr^{+6}) elimination was observed when the inoculum size, pH, incubation period, and agitation were at their middle values. The graph illustrates the trend of chromium (Cr^{+6}) elimination, which initially climbs, reaches a peak at the midpoint, and thereafter drops (Fig. 8- A, B).

Fig. 9 displays the ideal option for achieving maximal removal of chromium (Cr^{+6}), as determined by the Design-Expert software. The validation trials were carried out using shake flask experiments in triplicate, following the optimum solution parameters of 3.68 ml of bacterial cell inoculum size at a concentration of 10^8/ml, a pH of 6.8, an agitation speed of 104 rpm, and an incubation time of 90.13 min. The mean (70 percent) removal of chromium (Cr^{+6}) was discovered to fall within the 95 % expectation range of 70.56 %–77.79 % when the expected response data was compared with the results of the validation trial. Employing *B. mojavensis* C6, Abou et al. [38] have removed up to 83.3 % of the chromium (Cr^{+6}) from industrial wastewater; *Chang* et al. [39] discovered that bacterial isolates employing RSM BBD had an approximately 85 % Cr removal effectiveness. Another study by *Mat Arisah* et al. [29] found 85 % Cr

Table 6
CCD design layout of four coded factors with experimental and predicted response.

Run	Factor 1	Factor 2	Factor 3	Factor 4	Experimental Response	Predicted Response
	A: Inoculum size	B: pH	C: Incubation time	D: Agitation	Chromium reduction	
	min	N	N	%	%	%
1	5	8	120	0	41	41.21
2	3	2	72	75	12	13.00
3	5	8	24	150	45	47.04
4	7	6	72	75	46	44.17
5	1	4	120	0	25	21.54
6	3	6	72	75	65	67.83
7	3	6	168	75	42	46.50
8	3	10	72	75	30	32.33
9	5	4	24	0	26	28.71
10	5	4	24	150	31	30.63
11	3	6	72	75	66	67.83
12	5	4	120	0	26	26.79
13	3	6	72	0	32	31.50
14	3	6	72	75	68	67.83
15	1	4	24	150	20	18.38
16	1	8	120	150	45	40.88
17	3	6	72	225	46	49.83
18	1	4	120	150	36	35.46
19	3	6	72	75	70	67.83
20	5	8	120	150	62	60.63
21	5	8	24	0	44	42.63
22	0	6	72	75	10	15.17
23	1	8	120	0	26	24.46
24	3	6	72	75	70	67.83
25	3	6	72	75	68	67.83
26	1	8	24	0	21	21.88
27	5	4	120	150	46	43.71
28	3	6	00	75	32	30.83
29	1	4	24	0	20	19.46
30	1	8	24	150	26	23.29

Table-7
ANOVA for quadratic model.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9352.78	14	668.06	63.46	<0.0001	significant
A-Inoculum size	1261.50	1	1261.50	119.83	<0.0001	
B-pH	560.67	1	560.67	53.26	<0.0001	
C-Incubation time	368.17	1	368.17	34.97	<0.0001	
D-Agitation	504.17	1	504.17	47.89	<0.0001	
AB	132.25	1	132.25	12.56	0.0029	
AC	16.00	1	16.00	1.52	0.2366	
AD	9.00	1	9.00	0.8549	0.3698	
BC	0.2500	1	0.2500	0.0237	0.8796	
BD	6.25	1	6.25	0.5937	0.4530	
CD	225.00	1	225.00	21.37	0.0003	
A^2	2497.19	1	2497.19	237.20	<0.0001	
B^2	3497.19	1	3497.19	332.19	<0.0001	
C^2	1458.33	1	1458.33	138.52	<0.0001	
D^2	1265.19	1	1265.19	120.18	<0.0001	
Residual	157.92	15	10.53			
Lack of Fit	137.08	10	13.71	3.29	0.1004	not significant
Pure Error	20.83	5	4.17			
Cor Total	9510.70	29				
$\mathbf{R^2 = 0.9834}$	Pred$\mathbf{R^2}$ = 0.9138		Adj$\mathbf{R^2}$ = 0.9679		AdeqPrec = 23.89	

removal by *pseudomonas aeruginosa* which is quite high compared to our study due to environmental factors.

The elimination of chromium (Cr^{+6}) ions is significantly affected by a combination of inoculum size, agitation, incubation period and pH according to RSM employing CCD [10].

A vital component is time in the removal of chromium (Cr^{+6}). The capacity of *Pseudomonas aeruginosa* to eliminate different extra metal ions was contingent on time. The effectiveness of removal improves over time, and under specific circumstances, metal ions demonstrate optimal performance at a specific instant. Therefore, optimization is essential due to the variable influence of time on

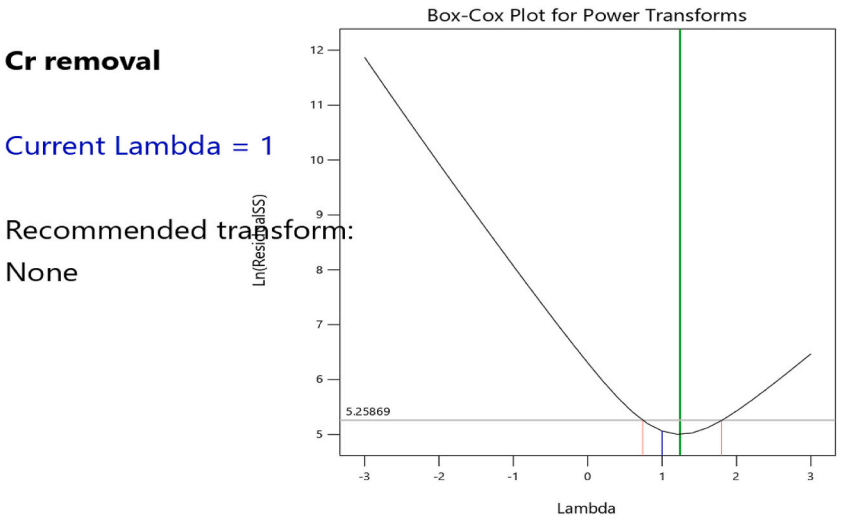


Fig. 7. Box-cox plot showing Lamda value for recommendation.

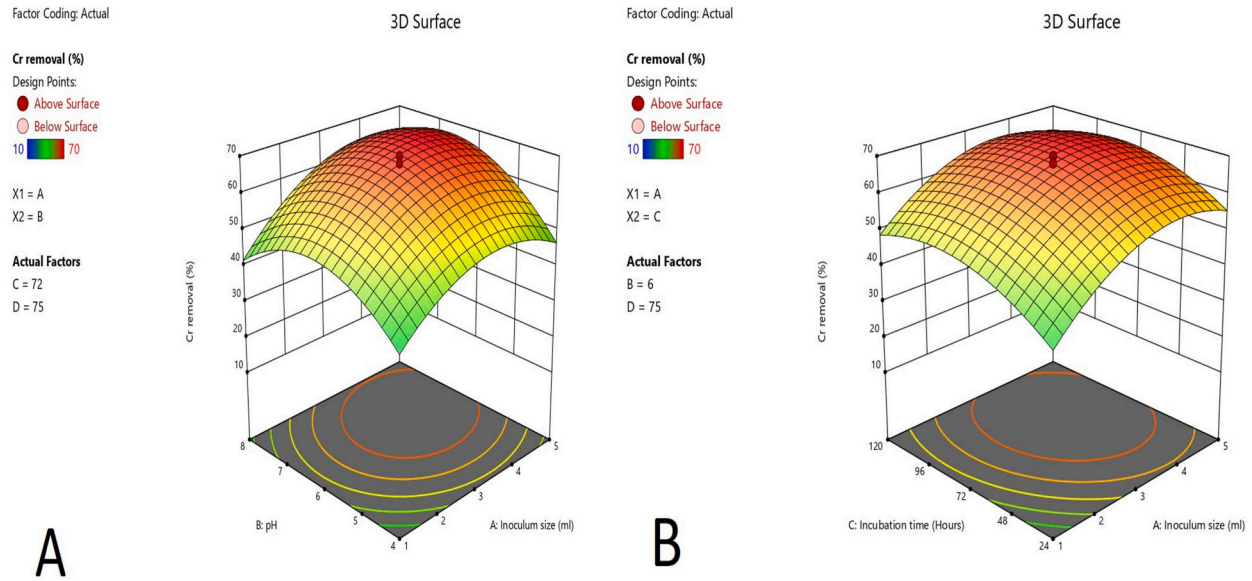


Fig. 8. The activity of Cr removal as a function of two-parameter interaction is displayed using three-dimensional surface plots.

different metals and bacteria. The optimal incubation period for maximum removal of chromium (Cr^{+6}) by *P. aeruginosa* is 90.13 min. However, if the contact time exceeds 90.13 min, the removal of chromium (Cr^{+6}) decreases. In a study conducted by *Singh R* [11] it was shown that the highest elimination of chromium is 80 % was reached after 95 h of growth employing *P. putida* under optimal circumstances. *Bandela* et al. [40] observed a greater reduction in heavy metal concentration after 72 h of incubation. Additionally, it has been discovered that the duration of the incubation has a notable impact, when it comes to cleaning up industrial effluent of metals.

pH is a crucial factor that affects bacterial metabolism and the molecular dynamics of solution-bound metal ions. Based on the latest study findings, it has been shown that the elimination of chromium (Cr^{+6}) is more effective at a pH of 6.8. There is a significant decrease in the exclusion of chromium (Cr^{+6}) when the pH of the medium varies. The bioremediation process can be influenced by changes in pH, which may result from fluctuations in the protonation of *Pseudomonas* ligands at the outermost layer of the cell. Changes in the external pH of the medium can have a considerable impact on the extent of ligand protonation, which refers to the binding of metal [41]. Sarin et al. [42] reported similar findings. At a pH of 6.8, it was observed that *Pseudomonas fluorescence* exhibited high efficacy in the removal of Cd. In a study, it was discovered that the optimal pH for bacterial species implicated in bioremediation of heavy metal is 7.0, which exceeds the current findings [9]. The pH throughout the bioremediation process had a significant impact on the solubility of metal ions. This was primarily influenced by the functional groups existing on the microbial cell [43]. Functional groups such as carboxyl, hydroxyl, phosphate, and amino groups have a notable impact on the absorption of heavy metals in

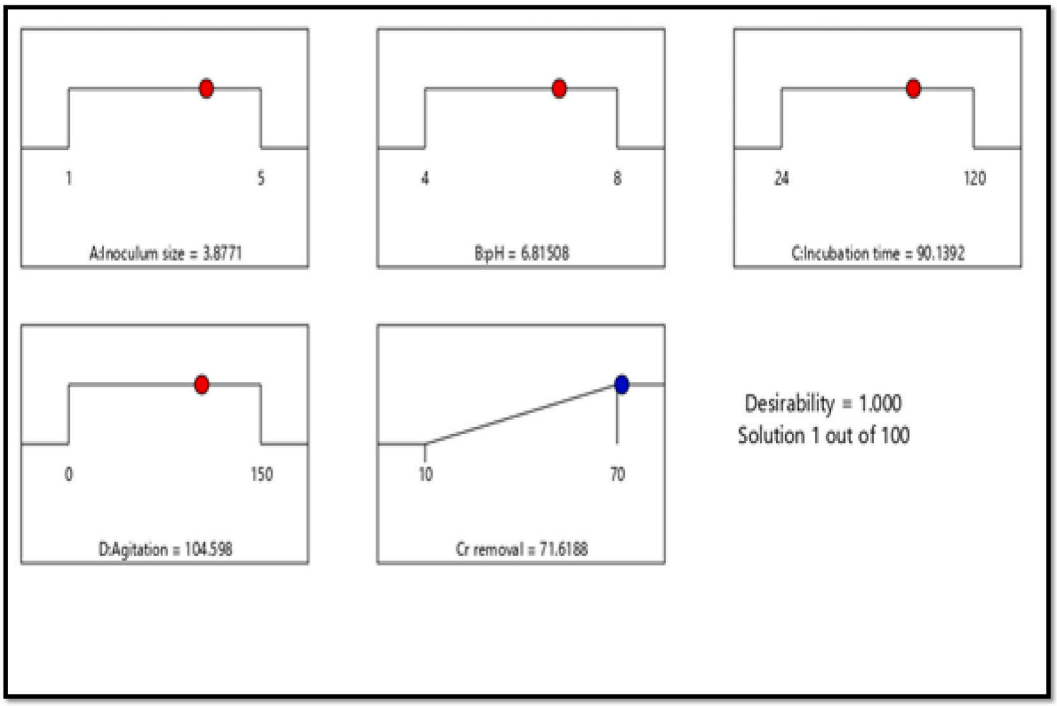


Fig. 9. Desirability plot displays the optimum solution recommended by the softwrae

microorganisms. The bearing of these functional groups is influenced by the pH level [44]. *Gabr* et al. [45] found that *P. aeruginosa PU21* maximum removed lead & nickel at pH 7. In contrast, *Lopez* et al. [46] found that pH 8.0 gives maximum removal of nickel by *P. aeruginosa*.

Bio removal of chromium (Cr^{+6}) [2] by the growth of *P. aeruginosa* was pointedly affected by the size of the primary inoculum. The concentration of chromium (Cr^{+6}) in the wastewater reduced by 71.61 % within a period of 90 h, despite the presence of 3.8 mL of inoculum containing 10^8 cells per milliliter. Furthermore, when the amount of inoculum was decreased, the duration for chromium (Cr^{+6}) reduction increased, and at the most favourable inoculum size, the duration dropped. Based on the findings of Mackey and Kerridge [47] and Robinson et al. [48], the size of the inoculum had a negligible effect on the elimination of Cr.

Agitation is the crucial factors in the chromium (Cr^{+6}) removal. The presence of dissolved oxygen can greatly influence the growth and multiplication of microorganisms, potentially leading to substantial changes in medium oxygen concentration [49], Fig. 8 depicts the influence of agitation on the ability of *P. aeruginosa* to reduce chromium (Cr^{+6}) by bio-reduction. Stirring at a speed of 104 revolutions per minute led to a decrease of 71 % in the concentration of chromium in its hexavalent form (Cr^{+6}). An observed reduction in chromium (Cr^{+6}) was seen under static settings; however, it was less apparent compared to shaking conditions. The results indicated that *P. aeruginosa* could be employed in both agitated and non-agitated conditions to reduce chromium (Cr^{+6}) levels in wastewater. Nevertheless, the rate of chromium (Cr^{+6}) reduction was slower when the circumstances were static compared to when they were shaking. Higher agitation speed leads to a more efficient removal of hexavalent chromium (Cr^{+6}). Nevertheless, the most effective elimination of chromium (Cr^{+6}) occurred when the speed reached an optimal level of 104 rpm. Chromium (Cr^{+6}) has a strong ability to dissolve in water, which means that residues or soil contaminated with chromium (Cr^{+6}) can readily be dissolved in liquid. Hence, *P. aeruginosa* possesses significant promise for the remediation of chromium (Cr^{+6}) pollution [9,50]. Tarangini et al. [51] discovered that the duration of agitation is a critical element in the process of biosorption of chromium by the robust microbes *Pseudomonas aeruginosa* and *Bacillus subtilis*.

4. Conclusion

This study on chromium removal by *P. aeruginosa* with optimization is significant because Chromium contamination in water sources is a significant environmental issue because of its poisonous nature and carcinogenicity. According to the current investigation, the most promising bacterial isolate, *Pseudomonas aeruginosa* shows efficient growth as well as tolerance up to 10,000 ppm of chromium (Cr^{+6}) and proved successful in eliminating chromium (Cr^{+6}) derived from water. Traditional microbiological techniques were used to identify *Pseudomonas aeruginosa* and confirmed by 16sRNA sequencing and Biolog analysis confirmed the metabolic system of isolate. An exploration of bioinformatics demonstrated that conserved domain of the laccase gene from bacterial isolates belonging to the multicopper oxidase superfamily. This finding further supports the presence of a conserved domain of laccase gene, which aids in the conversion of the highly toxic chromium (Cr+6) form of heavy metal to the less lethal chromium (Cr+3) form. The BioLog

metabolic research revealed that the *Pseudomonas aeruginosa* possessed a substantial ability to metabolize a wide range of complex carbohydrates. By employing the statistical techniques of PBD and CCD model, the optimization of numerous variables that influence microbial growth proves to be a valuable tool for predicting and understanding the interaction between independent variables such as agitation, pH, Incubation time, temperature, Inoculum size, and Initial concentration of metal ion, in order to achieve the maximum possible removal of chromium (Cr^{+6}). The model was employed to determine the optimal parameters: pH 6.8, an incubation time of 90 min, an inoculum size of 3.8 ml, and agitation at 104 rpm. The elimination of chromium (Cr^{+6}) was achieved with an efficacy of 71.61 % in these sceneries. The current study has demonstrated the high efficacy of *Pseudomonas aeruginosa JRHM33* in the removal of chromium (Cr^{+6}) from electroplating effluents. Therefore, this bacterium has the potential to be utilized for the treatment of industrial effluents containing chromium (Cr^{+6}) before they are discharged into bodies of water and they could lead to the expansion of more efficient, an economically efficient and ecologically sustainable system for treating wastewater. Utilizing microorganisms like *Pseudomonas aeruginosa* for chromium removal with optimization of process variables offers a promising avenue for bioremediation. Our future aspects of chromium bioremediation using *Pseudomonas* are developing genetically engineered *Pseudomonas aeruginosa JRHM33* strains with enhanced chromium (Cr^{+6}) reduction capabilities through metabolic engineering and the use of other chromium-reducing bacteria and using *Pseudomonas aeruginosa* consortia for more effective bioremediation by activating diverse chromium (Cr^{+6}) reduction pathways.

Jhn.

CRediT authorship contribution statement

Jayeshkumar R. Ruparelia: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Hiren K. Patel:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

References

[1] P. Neethu, M. Chinnamma, Natural adsorbents for agricultural waste water treatment, International Journal of Advanced Engineering, Management and Science 3 (4) (2017) 239823.
[2] M. Ahmaruzzaman, V.K. Gupta, Rice husk and its ash as low-cost adsorbents in water and wastewater treatment, Ind. Eng. Chem. Res. 50 (24) (2011) 13589–13613.
[3] P. Li, et al., Sources and consequences of groundwater contamination, Arch. Environ. Contam. Toxicol. 80 (2021) 1–10.
[4] H. Aydın, Y. Bulut, Ç. Yerlikaya, Removal of copper (II) from aqueous solution by adsorption onto low-cost adsorbents, J. Environ. Manag. 87 (1) (2008) 37–45.
[5] M. del Carmen Vargas-García, et al., Compost as a source of microbial isolates for the bioremediation of heavy metals: in vitro selection, Sci. Total Environ. 431 (2012) 62–67.
[6] S. Ye, et al., Co-occurrence and interactions of pollutants, and their impacts on soil remediation—a review, Crit. Rev. Environ. Sci. Technol. 47 (16) (2017) 1528–1553.
[7] P.X. Sheng, et al., Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: characterization of biosorptive capacity and investigation of mechanisms, J. Colloid Interface Sci. 275 (1) (2004) 131–141.
[8] S. Demim, et al., Study of heavy metal removal from heavy metal mixture using the CCD method, J. Ind. Eng. Chem. 20 (2) (2014) 512–520.
[9] S. Congeevaram, et al., Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates, J. Hazard Mater. 146 (1–2) (2007) 270–277.
[10] R. Kumar, et al., Response surface methodology approach for optimization of biosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp. Bacillus brevis, Chem. Eng. J. 146 (3) (2009) 401–407.
[11] R. Singh, R. Bhateria, Optimization and experimental design of the Pb2+ adsorption process on a nano-Fe3O4-based adsorbent using the response surface methodology, ACS Omega 5 (43) (2020) 28305–28318.
[12] A. Choińska-Pulit, J. Sobolczyk-Bednarek, W. Łaba, Optimization of copper, lead and cadmium biosorption onto newly isolated bacterium using a Box-Behnken design, Ecotoxicol. Environ. Saf. 149 (2018) 275–283.
[13] K. Gupta, C. Chatterjee, B. Gupta, Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India, Biologia 67 (2012) 827–836.
[14] X. Deng, P. Wang, Isolation of marine bacteria highly resistant to mercury and their bioaccumulation process, Bioresour. Technol. 121 (2012) 342–347.
[15] A. Cicatelli, et al., Genetic and biochemical characterization of rhizobacterial strains and their potential use in combination with chelants for assisted phytoremediation, Environ. Sci. Pollut. Control Ser. 24 (2017) 8866–8878.
[16] H. Patel, et al., A sustainable production of lignocellulolytic enzymes and value added metabolites from banana pseudostem waste by Bacillus wakoensis NAULH-4, Process Biochem. 105 (2021) 42–49.
[17] R.L. Plackett, J.P. Burman, The design of optimum multifactorial experiments, Biometrika 33 (4) (1946) 305–325.
[18] G. Baskar, S. Renganathan, Optimization of L-asparaginase production by Aspergillus terreus MTCC 1782 using response surface methodology and artificial neural network-linked genetic algorithm, Asia Pac. J. Chem. Eng. 7 (2) (2012) 212–220.
[19] S. Sharma, A. Malik, S. Satya, Application of response surface methodology (RSM) for optimization of nutrient supplementation for Cr (VI) removal by Aspergillus lentulus AML05, J. Hazard Mater. 164 (2–3) (2009) 1198–1204.
[20] W.E. Marshall, E.T. Champagne, W.J. Evans, Use of rice milling byproducts (hulls & bran) to remove metal ions from aqueous solution, Journal of Environmental Science & Health Part A 28 (9) (1993) 1977–1992.
[21] P. Chaudhary, et al., Optimization of chromium and tannic acid bioremediation by Aspergillus niveus using Plackett–Burman design and response surface methodology, Amb. Express 7 (1) (2017) 1–12.
[22] M.Z. Alam, A. Malik, Chromate resistance, transport and bioreduction by Exiguobacterium sp. ZM-2 isolated from agricultural soil irrigated with tannery effluent, J. Basic Microbiol. 48 (5) (2008) 416–420.
[23] R. Abou-Shanab, P. Van Berkum, J. Angle, Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of Alyssum murale, Chemosphere 68 (2) (2007) 360–367.

[24] A. Batool, et al., Isolation and screening of chromium resistant bacteria from industrial waste for bioremediation purposes, Braz. J. Biol. 83 (2021).
[25] J.R.M.B. Smily, P.A. Sumithra, Optimization of chromium biosorption by fungal adsorbent, Trichoderma sp. BSCR02 and its desorption studies, HAYATI Journal of Biosciences 24 (2) (2017) 65–71.
[26] N.P. Lotlikar, et al., Potential of marine-derived fungi to remove hexavalent chromium pollutant from culture broth, Indian J. Microbiol. 58 (2018) 182–192.
[27] S. Sharma, P. Malaviya, Bioremediation of tannery wastewater by chromium resistant novel fungal consortium, Ecol. Eng. 91 (2016) 419–425.
[28] V. Kumar, S. Dwivedi, Hexavalent chromium reduction ability and bioremediation potential of Aspergillus flavus CR500 isolated from electroplating wastewater, Chemosphere 237 (2019) 124567.
[29] F. Mat Arisah, et al., Bacterial Resistance against heavy metals in Pseudomonas aeruginosa RW9 involving hexavalent chromium removal, Sustainability 13 (17) (2021) 9797.
[30] V.Y. Shukla, D.R. Tipre, S.R. Dave, Optimization of chromium (VI) detoxification by Pseudomonas aeruginosa and its application for treatment of industrial waste and contaminated soil, Ann. Finance 18 (2) (2014) 128–135.
[31] F. Kafilzadeh, S. Saberifard, Isolation and identification of chromium (VI)-resistant bacteria from Soltan Abad river sediments (Shiraz-Iran), Jundishapur Journal of Health Sciences 8 (1) (2016).
[32] K. Pandian, A. Thatheyus, D. Ramya, Bioremoval of chromium, nickel and zinc in electroplating effluent by Pseudomonas aeruginosa, Open Journal of Water Pollution and Treatment 2014 (2) (2014) 75–82.
[33] Y. Tohsato, H. Mori, Phenotype profiling of single gene deletion mutants of E. coli using Biolog technology, Genome informatics 21 (2008) 42–52.
[34] D.J. Baumler, et al., Inferring ancient metabolism using ancestral core metabolic models of enterobacteria, BMC Syst. Biol. 7 (2013) 1–17.
[35] A. Alengebawy, et al., Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications, Toxics 9 (3) (2021) 42.
[36] M.A. Alshiekheid, et al., Biodegradation of chromium by laccase action of Ganoderma Multipileum, J. King Saud Univ. Sci. 35 (10) (2023) 102948.
[37] R. Dhingani, G. Shah, B. Joshi, Sequential optimization of bioprocess nutritional parameters for maximum l-asparaginase production from Pseudomonas aeruginosa BGR1I1, Biocatal. Biotransform. 41 (3) (2023) 198–207.
[38] Abou-Taleb, A., S.S. Abd El-Salam, and O.T. Helmy, Bioremediation of Cr (VI) from industrial effluents using Bacillus mojavensis C6: process of optimization and application Reyad M. El-Sharkawy1, AlShaymaa I. Ahmed2, Mohamed O. Abdel-Monem1, Khadiga.
[39] J. Cheng, et al., Optimization of hexavalent chromium biosorption by Shewanella putrefaciens using the Box-Behnken design, Water, Air, Soil Pollut. 232 (2021) 1–14.
[40] N. Bandela, et al., Heavy metal removal by isolates from domestic and industrial waste water, J. Mater. Environ. Sci. 7 (12) (2016) 4589–4595.
[41] V. Singh, et al., Isolation and characterization of Pseudomonas resistant to heavy metals contaminants, Int. J. Pharmaceut. Sci. Rev. Res. 3 (2) (2010) 164–167.
[42] C. Sarin, S. Sarin, Removal of cadmium and zinc from soil using immobilized cell of biosurfactant producing bacteria, Environment (Wash. D C) 3 (2) (2010) 49–53.
[43] T.M. Palanivel, et al., Bioremediation of copper by active cells of Pseudomonas stutzeri LA3 isolated from an abandoned copper mine soil, J. Environ. Manag. 253 (2020) 109706.
[44] A. Chergui, et al., Simultaneous biosorption of Cu2+, Zn2+ and Cr6+ from aqueous solution by Streptomyces rimosus biomass, Desalination 206 (1–3) (2007) 179–184.
[45] R. Gabr, S. Hassan, A. Shoreit, Biosorption of lead and nickel by living and non-living cells of Pseudomonas aeruginosa ASU 6a, Int. Biodeterior. Biodegrad. 62 (2) (2008) 195–203.
[46] A. Lopez, et al., Effect of pH on the biosorption of nickel and other heavy metals by Pseudomonas fluorescens 4F39, J. Ind. Microbiol. Biotechnol. 24 (2) (2000) 146–151.
[47] B. Mackey, A. Kerridge, The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef, Int. J. Food Microbiol. 6 (1) (1988) 57–65.
[48] T.P. Robinson, et al., The effect of inoculum size on the lag phase of Listeria monocytogenes, Int. J. Food Microbiol. 70 (1–2) (2001) 163–173.
[49] J.X.W. Hay, et al., Optimized Growth of Rhodobacter Sphaeroides OU 001 Using Response Surface Methodology (RSM), 2012.
[50] A. Rehman, et al., Chromium tolerance and reduction potential of a Bacillus sp. ev3 isolated from metal contaminated wastewater, Bull. Environ. Contam. Toxicol. 81 (2008) 25–29.
[51] K. Tarangini, et al., Statistical optimization of process parameters for Cr (VI) biosorption onto mixed cultures of Pseudomonas aeruginosa and Bacillus subtilis, Clean: Soil, Air, Water 37 (4-5) (2009) 319–327.