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ORIGINAL ARTICLE

Mechanistic action of lead removal by the native isolate *Bacillus amyloliquefaciens* ON261680.1

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Abstract Lead (Pb) is one of the substantial hazard pollutants that cause soil pollution. Bioremediation is an eco-friendly and cost-effective technique that provides a convenient solution for lead removal. In the present study, lead resistant bacterial strains were isolated from industrial effluents contaminated soil with lead minimum inhibitory concentrations reached 15000 ppm. The most resistant isolate was identified by 16S rRNA sequencing as *Bacillus amyloliquefaciens* ON261680.1. Taguchi statistical design was applied to optimize the environmental and nutritional factors that lead to maximum lead removal. The optimized conditions for the highest lead removal of lead concentration, incubation temperature, incubation time, peptone, yeast, beef extract, NaCl concentrations, pH, inoculum size and inoculum age were 10000 ppm, 30 °C, 96 hrs incubation, 0.5 g, 0.5 g, 1.5 g, 6 g, 9, 15 mL and 48 hrs old culture, respectively. The most significant factors ($p < 0.05$) were incubation time, pH, lead concentration, and incubation temperature. Mechanistic action was investigated through employing Fourier transform infrared spectroscopy (FTIR), Energy-dispersive X-ray spectroscopy (EDX) and transmission electron microscopy (TEM). It was revealed that *Bacillus amyloliquefaciens* ON261680.1 removed the lead pollutant through bio-adsorption, deposition and complexation on the cell surface. The present study may pave the way to use *Bacillus amyloliquefaciens* ON261680.1 as an efficient bioremediation tool.

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1. Introduction

Heavy metal pollution was considered as hazardous dilemma that influencing aquatic and terrestrial life (Kalaimurugan et al., 2020). Lead (Pb) is one of the most widely distributed contaminants present in soil, water, and air (Kushwaha et al., 2018) due to its economic importance in several industries e.g., batteries, pigments, metal smelting, the production of lead arsenate pesticides and lead-containing water pipes (Hanfi et al., 2020). Lead pollution can result in reversible injury to the central nervous, hematopoietic, hepatic, and renal systems failures (Patel et al., 2018).

Bacteria possess several metabolism-dependent and metabolism-independent methods for the uptake and accumulation of toxic metals. Bioremediation is an approach for metal recovery and remediation is the elimination of these hazardous metals from soil, sludge, and wastewater via microbe-based technology (Manoj et al., 2020). Microorganisms possess several cellular mechanisms for efflux, extracellular sequestration, metal modification, and precipitation of Lead ions, among others (Pratush et al., 2018). The microbial cells might potentially modify their shape to promote the generation of the siderophores to support the intracellular bioaccumulation of Lead (Sharma et al., 2018). *Bacillus amyloliquefaciens* has been used as a biocontrol agent (Ngalimat et al., 2021) to enhance plant growth directly (by nitrogen fixation, phytohormone production and phosphate solubilization) or indirectly by improving the plant health (Chowdhury et al., 2015). The forceful effect of *Bacillus amyloliquefaciens* in promoting plant growth activity as well as organophosphorus pesticide (OP) removal, paved the way for more recent investigations of the possible use of *Bacillus amyloliquefaciens* in agricultural and environmental remediations (Meng et al., 2019). Moreover, Maarof et al. (2018) reported that *Bacillus amyloliquefaciens* strain KIK-12 can effectively reduce molybdate to molybdenum with optimum molybdate concentration ranging between 30 and 50 mM.

The adsorption process is a metabolically independent method of heavy metal accumulation that relies mostly on surface features, such as some main functional groups present on the cell membrane and electrostatic interactions (Zabochnicka-witek & Krzywinos, 2014). The process of adsorption is also impacted by numerous elements like pH, temperature, ionic strength, absorbent quantity, and size, etc., (Timkova et al., 2018). Teng et al. (2019) isolated and identified some potent strains of inorganic phosphate-solubilizing bacteria (iPSB) capable of immobilizing lead by dissolving insoluble phosphate components. In the present study, native lead resistant microorganisms were isolated from industrial effluents contaminated soil and screened for their bioremediation potential. The most potent lead resistant microorganism was identified and statistically optimized using Taguchi design to maximize the lead removing efficiency. Moreover, mechanistic studies of the possible bioremediation mechanisms were investigated (Fig. 1).

2. Materials and methods

2.1. Sample collection

Soil samples were obtained from industrial effluents polluted area in Alexandria, Egypt. All soil samples were stored in labelled sterile containers before being delivered to the laboratory for analysis (Kang et al., 2015).

2.2. Isolation of lead resistant bacteria

The lead resistant bacteria were isolated from soil samples using serial dilution technique where 1 g soil was suspended in sterilized distilled water (10 mL), vortexed for 10 min then serially diluted up to 10^{-6} dilutions. 100 µL of each dilution were distributed over lead-containing (100 ppm) nutrient agar medium using spread plate technique. The plates were incubated for 48 hrs at 37 °C. Single colonies were purified through streaking technique over lead-containing (100 ppm) nutrient agar medium (Dey et al., 2016).

2.3. Detection of the minimum inhibitory concentration of lead

The minimal inhibitory concentration (MIC) of lead was assessed using serial dilutions ranged from 1000 to 25000 ppm. Nutrient agar plates were supplemented with

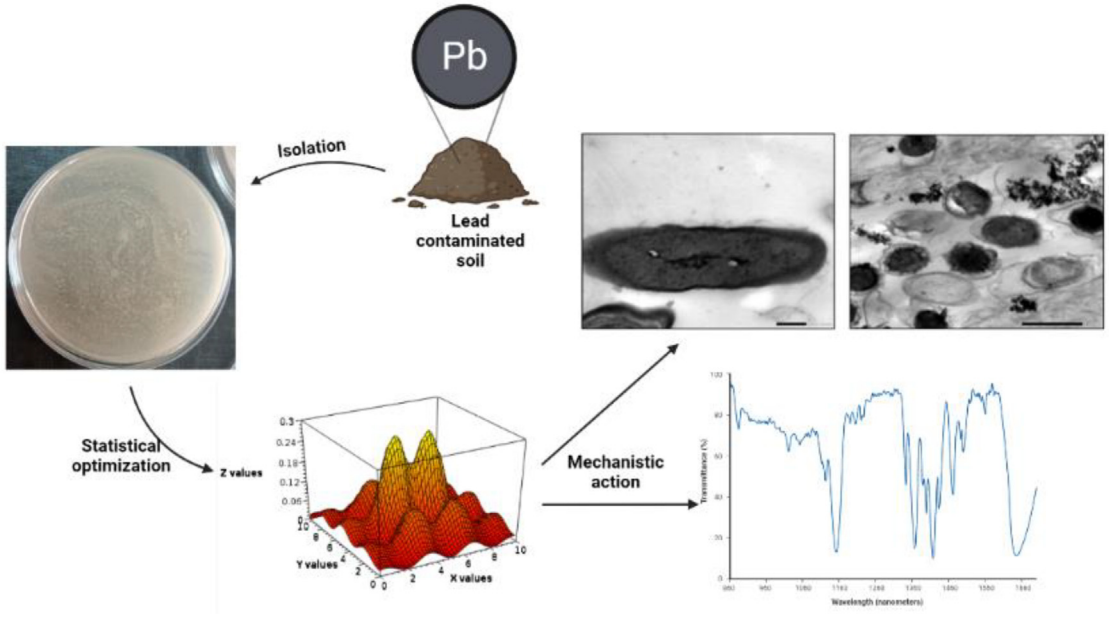

Fig. 1 Graphical presentation of the study design.

100 µL of each lead concentration. 0.5 McFarland of lead-resistant bacterial suspensions (1.5×10^8 CFU/mL) was adjusted (OD 800 nm) then, 20 µL of each suspension was streaked over the lead-supplemented nutrient agar plates surface. The inoculated plates were daily observed for a week to assess the bacterial growth. The minimum inhibitory concentration was defined as the lowest concentration that inhibited the visible microbial growth (Nokman et al., 2019). A single colony of the most promising bacterial strain with the highest MIC value was selected and identified by 16S rRNA gene sequencing (Sharma & Shukla, 2021). The bacterial DNA was extracted and amplified against forward (5-AGTTT GATCCTGGCTCAG-3) and reverse (5-GGCTTACCTTGT TACGACTT-3) 16S rRNA primers. Multiple sequence alignment was performed after sequencing the 16S rDNA in accordance with the National Center for Biotechnology Information (NCBI) database. Finally, the phylogenetic tree of the most resistant lead isolate was created through distance matrix analysis using the NT system (Shahin et al., 2022).

2.4. Bacterial lead removal optimization using experimental Taguchi statistical design

Qualitek-4 software (V. 14.5, Nutek Inc., MI, USA) was used to design Taguchi statistical experiments for optimizing the environmental (lead concentration, pH, incubation temperature, inoculum size, inoculum age and incubation time) and nutritional (peptone, beef extract, yeast extract, NaCl) factors to maximize the bacterial bioremediation of lead (Taran et al., 2015). Table 1 showed the factors and their levels, while Table 2 showed the experimental matrix. Each run was prepared and conducted according to the experimental Taguchi statistical design (Table 2) then at end of each experiment, flasks were centrifuged (12000 rpm for 5 min) and the supernatants were assessed for lead concentration using atomic absorption spectrophotometry. All the experiments were done in triplicates (Taran et al., 2013).

2.5. Statistical analysis

Taguchi DOEs results were statistically analyzed using fixed-effects model of analysis of variance (ANOVA) in Qualitek-4 software (Taran et el., 2015).

2.6. Mechanistic action of the Pb removal

In order to determine the possible bio-adsorption mechanism, cells before (control) and after Pb^{2+} exposure was collected by centrifugation at 940 × g for 3 min then the bacterial pellets were washed twice with PBS buffer (Zhu et al., 2019), and studied using transmission electron microscope (TEM) (JEM-100 CX Joel).

Moreover, the control, the treated cells and the supernatant were lyophilized and Fourier-transform infrared spectroscopy (FTIR) (Perkin Elmer, Inc., Waltham, MA, USA) was applied to study the variations of functional groups on the bacterial cell surfaces. Lyophilized samples were studied through energy-dispersive X-ray spectroscopy (EDX) to determine the Pb content% in the prepared samples (control, treated supernatants and treated cell pellets).

2.6.1. Pb compound characterization

The untreated (control) and Pb^{2+} ions treated cells were collected, washed with deionized distilled water, lyophilized and subjected to X-Ray diffraction (XRD) analysis (XRD-BRUKER AXS, Cu Kα radiation source (λ = 0.154 nm), scan rate = 10°/ min).

3. Results and discussion

3.1. Isolation of lead-resistant bacterial colonies

From lead-contaminated soils, certain lead-resistant bacterial strains were identified. The microbial communities in the examined soils evolved a tolerance mechanism to hazardous chemicals (Wang et al., 2018). Five distinct organisms were chosen for further study from various colonies found on nutrient agar plates treated with 100 ppm lead.

3.2. Determination of lead MIC

Maximum lead tolerance was 15000 ppm for isolate 2 followed by 1000, 5000, 5000 and 10000 ppm for isolates 3, 5, 1 and 4 respectively. Therefore isolate 2 was selected and identified (as the highest lead resistant isolate) using 16 s rRNA profile. Similarly, Rittmann (2004) mentioned that *Aspergillus niger* RH 17 showed the highest lead resistivity reaching 15000 ppm when grown on lead-supplemented agar plates.

Table 1 Environmental and nutritional factors and their levels using Taguchi DOEs.

	Factor	Level 1	Level 2	Level 3
X1	Incubation time (h)	48	72	96
X2	pH	5	7	9
X3	Lead concentration (ppm)	5000	10,000	15,000
X4	Incubation temperature (°C)	30	35	40
X5	Inoculum size (ml)	5	10	15
X6	Inoculum age (h)	24	48	72
X7	Peptone (g)	0.5	1.5	2.5
X8	Beef extract (g)	0.5	1.5	2.5
X9	Yeast extract (g)	0.5	1.5	2.5
X10	NaCl (g)	4	5	6

However, *Acinetobacter* strain DF4/PUTK2 showed increased reduction percentage with increased concentration of lead up to 15000 ppm, while 30000 ppm reported a lethal effect (Muhammad et al., 2021). Ansari and Malik, (2007) reported the lead MIC value of *Bacillus* spp. as 1600 ppm. On the other hand, Raja et al. (2006) stated that *Bacillus* spp. could resist lead concentration up to 8000 ppm.

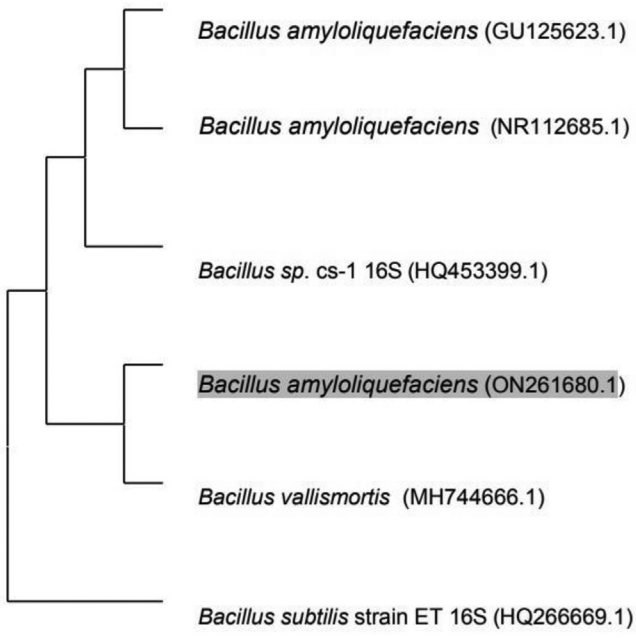

Fig. 2 Phylogenetic tree of *Bacillus amyloliquefaciens* ON261680.1.

3.3. Identification of isolate 2 using 16S rRNA profile

Isolate 2 was selected and identified as *Bacillus amyloliquefaciens* with 99.3% similarity and then deposited at GenBank with accession number ON261680.1. Fig. 2 showed the phylogenetic tree of *Bacillus amyloliquefaciens* ON261680.1.

3.4. Bioremediation of lead according to DOEs of environmental and nutritional factors

Optimum temperature, pH, concentration, inoculum size, inoculum age, incubation time, peptone, yeast extract, beef extracts and NaCl concentrations were evaluated using Taguchi optimization matrix demonstrated 27 orthogonal arrays (Table 2) with regression equation:

$$\begin{aligned} Remaininglead = {} & -56.1 - 0.819X1 - 8.56X2 \\ & + 0.01876X3 + 3.03X4 - 0.81X5 \\ & - 0.093X6 + 1.89X7 + 0.88X8 \\ & + 1.92X9 + 0.59X10 \end{aligned} \quad (1)$$

In the regression equation (Eq. (1), the positive values signify the positive effect of the tested variables while the negative values indicate their inverse effect on the response (remaining lead concentration) (Omidi et al., 2017). Data in Table 3 showed that the highest bioremediation of lead by *Bacillus*

Table 2 Experimental matrix of environmental and nutritional factors using Taguchi DOEs.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	2	2	2	2	2	2
3	1	1	1	1	3	3	3	3	3	3
4	1	2	2	2	1	1	2	2	2	2
5	1	2	2	2	2	2	3	3	3	3
6	1	2	2	2	3	3	1	1	1	1
7	1	3	3	3	1	1	1	3	3	3
8	1	3	3	3	2	2	2	1	1	1
9	1	3	3	3	3	3	3	2	2	2
10	2	1	2	3	1	2	3	1	2	3
11	2	1	2	3	2	3	1	2	3	1
12	2	1	2	3	3	1	2	3	1	2
13	2	2	3	1	1	2	3	2	3	1
14	2	2	3	1	2	3	1	3	1	2
15	2	2	3	1	3	1	2	1	2	3
16	2	3	1	2	1	2	3	3	1	2
17	2	3	1	2	2	3	1	1	2	3
18	2	3	1	2	3	1	2	2	3	1
19	3	1	3	2	1	3	2	1	3	2
20	3	1	3	2	2	1	3	2	1	3
21	3	1	3	2	3	2	1	3	2	1
22	3	2	1	3	1	3	2	2	1	3
23	3	2	1	3	2	1	3	3	2	1
24	3	2	1	3	3	2	1	1	3	2
25	3	3	2	1	1	3	2	2	2	1
26	3	3	2	1	2	1	3	3	3	2
27	3	3	2	1	3	2	1	1	1	3

amyloliquefaciens ON261680.1 was 99.840 % at 30 °C, 10000 ppm, pH 9, 96 h incubation, 15 mL inoculum size, 48 h inoculum age, 0.5 g peptone, 0.5 g yeast extract, 1.5 g beef extract and 6 g NaCl. Fig. 3 showed the interactions between the significant factors (concentration, temperature, pH time). Raja et al. (2006) revealed that *Pseudomonas* BC15 and *Bacillus* sp. J1 showed 65 and 75% Lead accumulation respectively. However, Wang et al. (2018) reported that heavy-metal uptake is an exothermic reaction, and the temperature elevation destroys the metal uptake sites on the surface of the bacterial cell wall or induces ion exudation into solution by bacteria. Gómez-Ramírez et al. (2015) studied *Microbacterium oxydans* CM3 and CM7 Pb-bioremediation optimization potential using response surface methodology (RSM). The optimum conditions for Pb removal by strain CM3 was observed at pH 6.81, temperature 35.31 °C, the Pb concentration of 337.35 mg/L, with 60.42% removal. However, strain CM7 showed maximum Pb remediation at temperature 35.65, pH 5.28, the Pb concentration of 346.95 mg/L and the Pb removal was 59.05%.

3.5. Statistical analysis of Taguchi DOEs

Statistical analysis was done using fixed-effects model of analysis of variance (ANOVA) in Qualitek-4 software. Analysis of variance showed that time, pH, concentration and temperature were the significant factors (p-value < 0.05) while inoculum size, inoculum age, peptone, beef extract, yeast extract and NaCl were not significant (p-value > 0.05) **(**Fig. 3**a,b,c,d and 4b)**. Metal biosorption is a complex process includes ion exchange, precipitation and complexation **(**Meng et al., 2020**).** Analysis of variance (ANOVA) results (Table 4) are based on calculation correspond to sum of the squares which applied for calculation of factors effect, Fisher's F-ratios and P-values were also represented. The normal probability of residuals **(**Fig. 4**c)** indicates almost no serious destruction of the DOE assumptions. The model summary presents high R^2-value of 93.65% for Pb removal and specifies that there is agreement between the experimental and predicted outcomes. Satisfactory normal distribution of the results confirms the normality assumptions made earlier and the independence of the residuals **(**Dil et al., 2016**)**. The results correspond to plotting residual values vs. the number of experiments **(**Fig. 4**a)** shows random distribution of the residual values around zero and proof accuracy of the selected model **(**Asfaram et al., 2016**)**.

Model Summary.

S	R-sq	R-sq(adj)	R-sq(pred)
27.2669	93.65%	89.68%	82.61%

3.6. Mechanistic action of the Pb removal using Bacillus amyloliquefaciens ON261680.1

EDX analysis of untreated control supernatant revealed the high presence of Pb ions while no traces of Pb ions were noticed in the biologically treated supernatant sample **(**Fig. 5**a and c).** It is worth noting that Pb was present on the

Table 3 Taguchi DOEs and corresponding bioremediation of lead by *Bacillus amyloliquefaciens* ON261680.1.

	Time (h)	pH	Conc (ppm)	Temp (°C)	Inoculum size (ml)	Inoculum age (h)	Peptone (g)	Beef extract (g)	Yeast extract (g)	NaCl (g)	Remaining lead (ppm)
1	48	5	5000	30	5	24	0.5	0.5	0.5	4	44.56
2	48	5	5000	30	10	48	1.5	1.5	1.5	5	48.256
3	48	5	5000	30	15	72	2.5	2.5	2.5	6	41.97
4	48	7	10,000	35	5	24	0.5	1.5	1.5	5	129.4
5	48	7	10,000	35	10	48	1.5	2.5	2.5	6	121.9
6	48	7	10,000	35	15	72	2.5	0.5	0.5	4	113.837
7	48	9	15,000	40	5	24	0.5	2.5	2.5	6	236.3
8	48	9	15,000	40	10	48	1.5	0.5	0.5	4	221.4
9	48	9	15,000	40	15	72	2.5	1.5	1.5	5	228.1
10	72	5	10,000	40	5	48	2.5	0.5	1.5	6	124.27
11	72	5	10,000	40	10	72	0.5	1.5	2.5	4	112.28
12	72	5	10,000	40	15	24	1.5	2.5	0.5	5	132.8
13	72	7	15,000	30	5	48	2.5	1.5	2.5	4	229.9
14	72	7	15,000	30	10	72	0.5	2.5	0.5	5	210.22
15	72	7	15,000	30	15	24	1.5	0.5	1.5	6	209.9
16	72	9	5000	35	5	48	2.5	2.5	0.5	5	33.99
17	72	9	5000	35	10	72	0.5	0.5	1.5	6	30.93
18	72	9	5000	35	15	24	1.5	1.5	2.5	4	32.36
19	96	5	15,000	35	5	72	1.5	0.5	2.5	5	228.51
20	96	5	15,000	35	10	24	2.5	1.5	0.5	6	227.344
21	96	5	15,000	35	15	48	0.5	2.5	1.5	4	212.22
22	96	7	5000	40	5	72	1.5	1.5	0.5	6	28.33
23	96	7	5000	40	10	24	2.5	2.5	1.5	4	25.35
24	96	7	5000	40	15	48	0.5	0.5	2.5	5	29.37
25	96	9	10,000	30	5	72	1.5	2.5	1.5	4	34.33
26	96	9	10,000	30	10	24	2.5	0.5	2.5	5	30.47
27	96	9	10,000	30	15	48	0.5	1.5	0.5	6	16.0

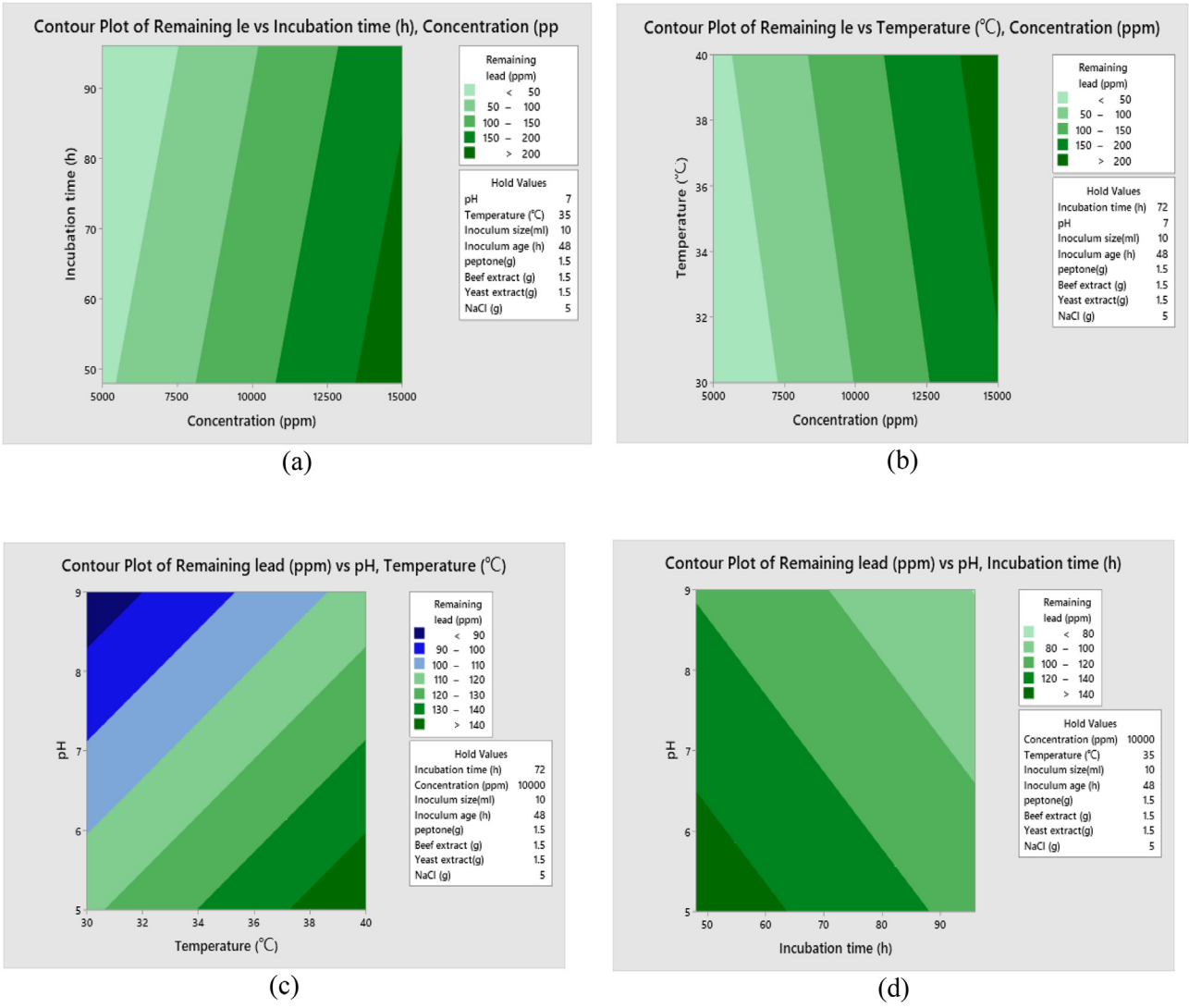

Fig. 3 Surface plot of nutritional and environmental factors using Taguchi DOEs: remaining lead versus initial lead concentration and incubation time (a), remaining lead versus initial lead concentration and temperature (b), remaining lead versus pH and temperature (c) and remaining lead versus pH and incubation time (d).

Table 4 Analysis of variance of Taguchi DOEs.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	10	175,343	17,534	23.58	0.000
Time	1	6954	6954	9.35	0.008
pH	1	5282	5282	7.10	0.017
CONC	1	158,443	158,443	213.11	0.000
TEMP	1	4128	4128	5.55	0.032
Inoculum size	1	296	296	0.40	0.537
Inoculum age	1	89	89	0.12	0.734
Peptone	1	64	64	0.09	0.773
Beef extract	1	14	14	0.02	0.893
Yeast extract	1	66	66	0.09	0.769
NaCl	1	6	6	0.01	0.927
Error	16	11,896	743		
Total	26	187,238			

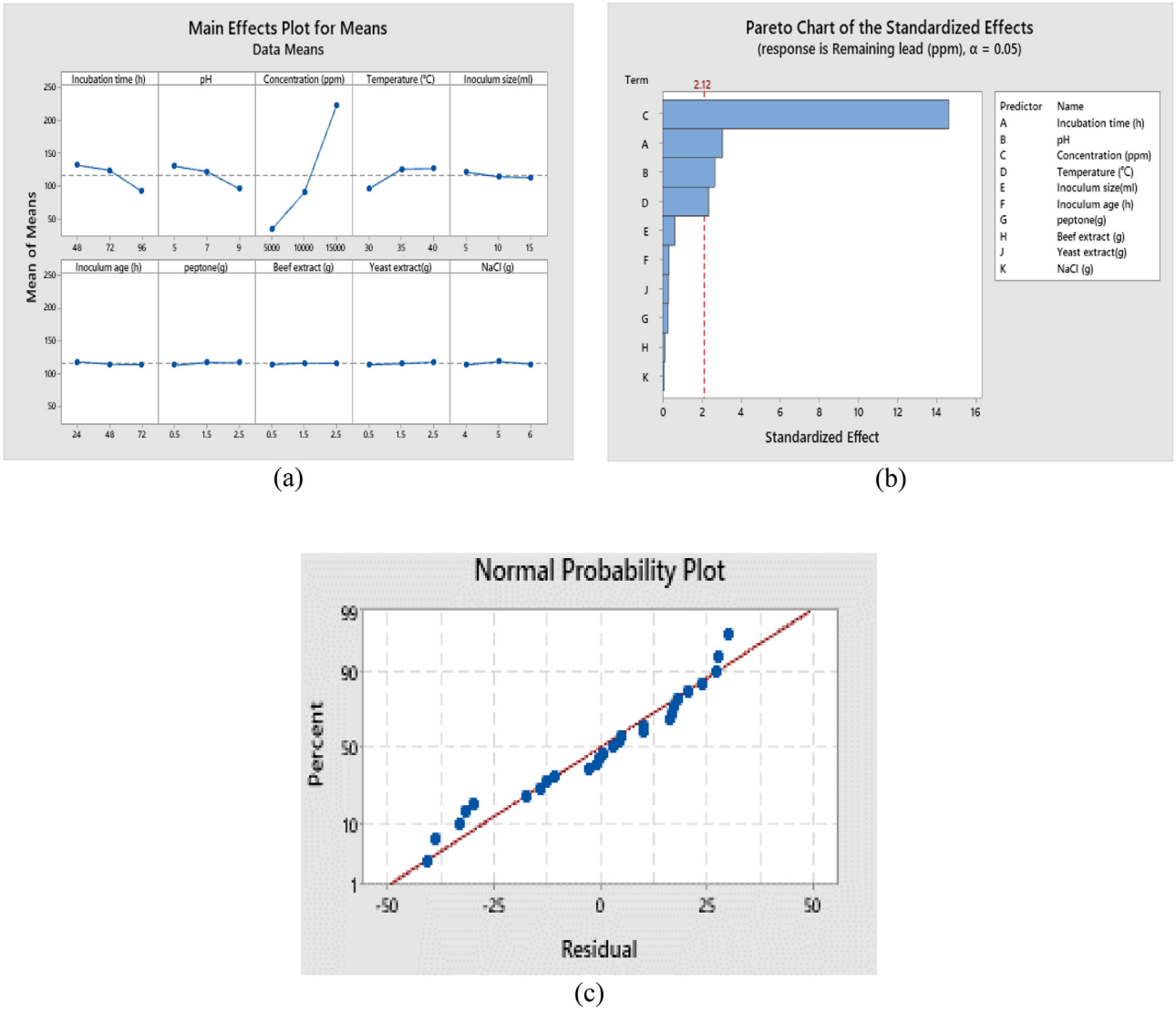

Fig. 4 Main effects of the studied variables (a), Pareto chart (b) and normal plot of the optimized lead removal using Taguchi DOEs.

treated bacterial cell surface **(**Fig. 5**b)** which clearly indicates the possible bio-adsorption of Pb ions with possible formation of Pb-containing compounds on *Bacillus amyloliquefaciens* ON261680.1 cells surface. Chen et al. (2016) suggested the possible mechanism behind the observed lead precipitation as insoluble complexes on the microbial cell surface that can be attributed to the presence of phosphate, amine, hydroxyl and carboxyl groups which were served as metal attachment sites on the cell surface. Consistently, the present study supported that several functional groups of the cell wall (e.g., O–H, N–H, amide C = O and P-O as indicated by FTIR, Fig. 7) provide a net overall negative charge to the cell surface, and concurrently a high binding affinity for Pb^{2+} metal cations via counterion interactions. At the same time, the presence of single pair electrons on N and O delocalizes into the 6p empty orbit of Pb^{2+} and can form coordination bond. (Fig. 9).

Transmission electron microscopy (TEM) was applied to *Bacillus amyloliquefaciens* ON261680.1 control cells before and after incubation with lead using the optimum conditions. Fig. 6**A** indicated that the control bacterial cells showed typical intact cell with native morphology. While Fig. 6**B** showed lead bio-adsorption inside the *Bacillus amyloliquefaciens* ON261680.1 cells. Zhu et al. (2022) mentioned that the surface of *Acinetobacter calcoaceticus*/IBWS700 appeared stacked, wrinkled, and shortened with the appearance of a large number of stacks after Pb adsorption, which may be due to these reasons, i, physical adsorption related to the special surface and the microporous structure of the extracellular polysaccharides secreted by iPSB20–3; ii, electrostatic adsorption dependent on the pH; iii, complexation, substitution, and cation-π interactions between Pb and functional groups on the IBWS700 surface **(**Deng et al., 2019**)**.

FTIR spectra was executed to assess whether the binding of Pb(II) ions to *Bacillus amyloliquefaciens* ON261680.1 cells involved the surface functional groups. Due to the interaction of Pb(II) ions with the surface functional groups, the peaks may shift to lower or higher wavenumbers after lead loading. The spectra are depicted in Fig. 7 and the corresponding results are listed in Table 5. The spectra confirmed alterations of molecular vibrations of specific functional groups in the

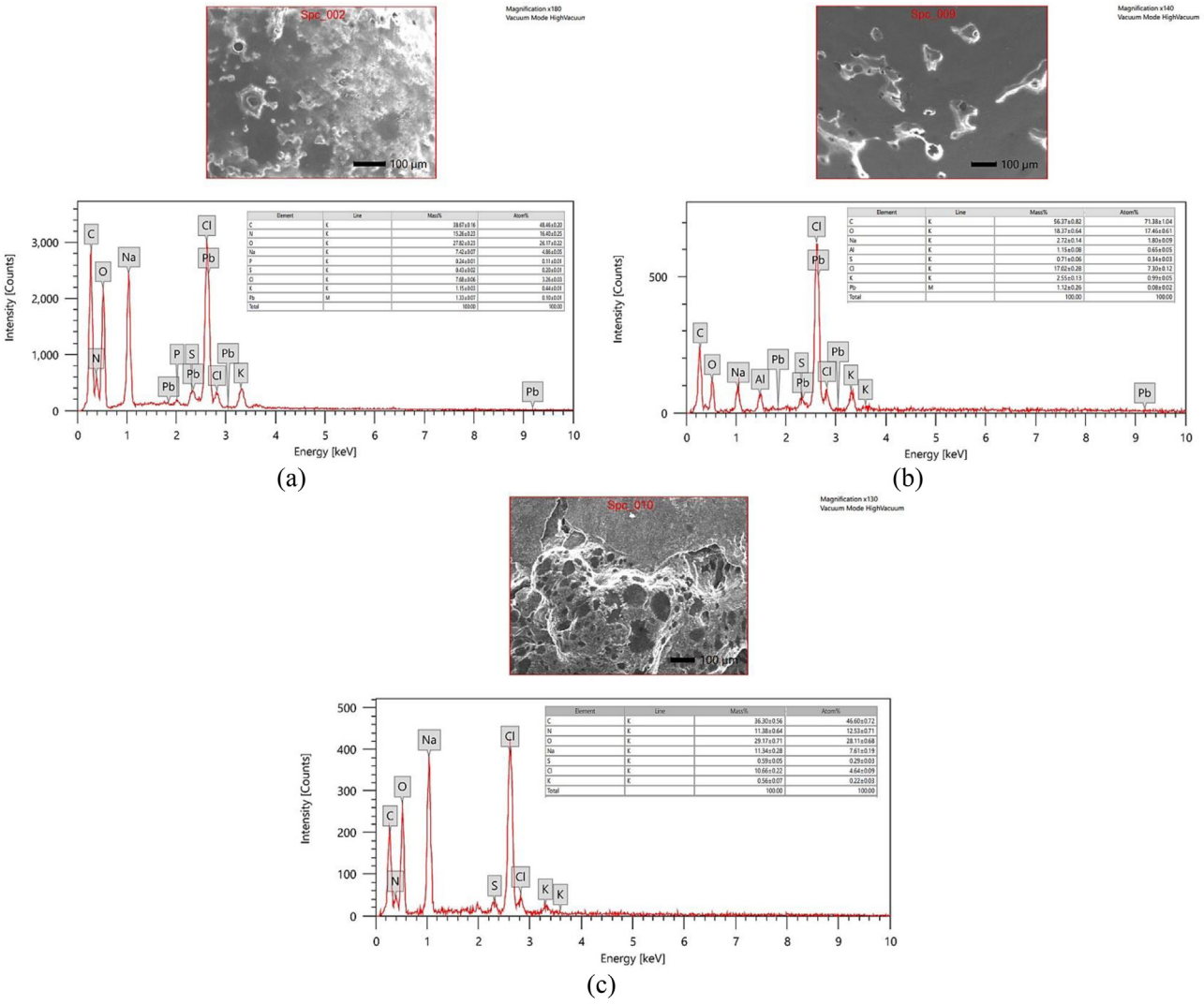

Fig. 5 EDX analysis of control untreated culture (a), bacterial cell surface (b) and cultural supernatant (c) after treatment.

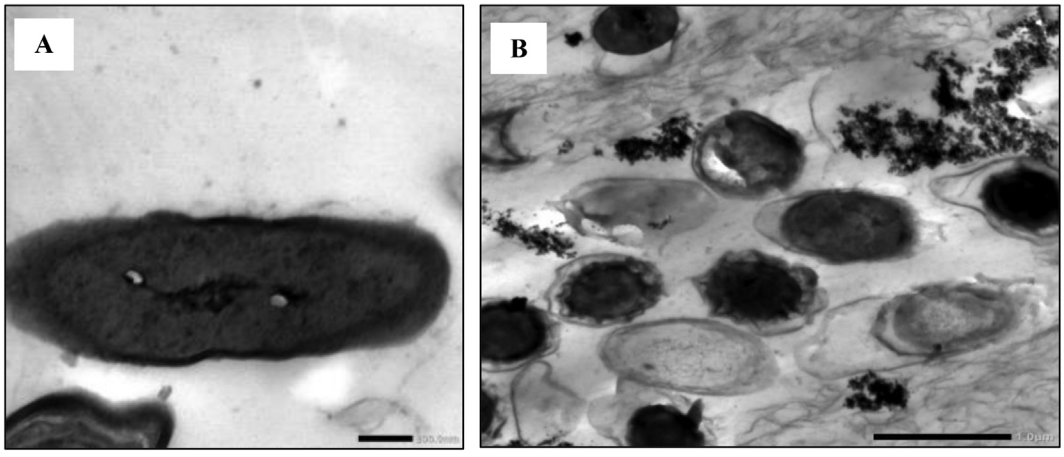

Fig. 6 TEM examination of *Bacillus amyloliquefaciens* ON261680.1 control cells (A) and after incubation with lead (B).

bacterial cell surface before and after adsorption of Pb(II). The *Bacillus* cell walls are known to contain large amounts of anionic polymers, teichoic acids and teichuronic acids (Tempest et al., 1968). The distinct absorption bands before and after

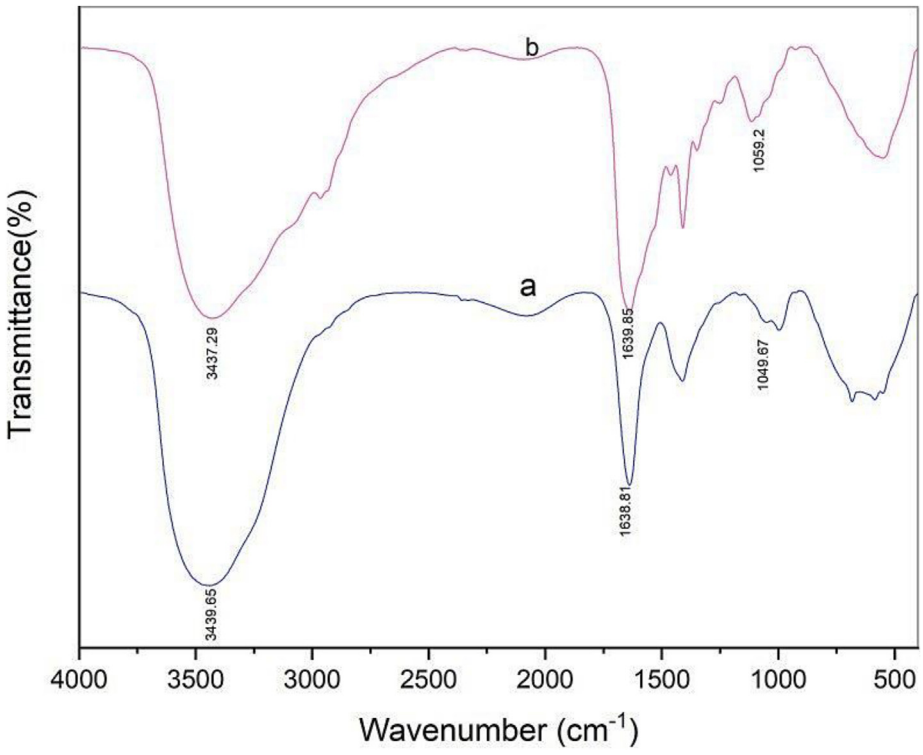

Fig. 7 FTIR analyses of bacterial cells before (blue) and after (red) treatment with Pb.

the adsorption process are corresponding to the stretching vibrations of O–H, N–H, amide C = O and P-O of PO_4^{-3}. These absorption bands (Table 5) were shifted after adsorption of Pb(II) ions as shown in Fig. 7. It was found that the stretching vibration bands of O–H and N–H bonds were shifted by 2.36 cm^{-1} to higher wavenumbers. This shifting of peaks to higher wavenumbers can be attributed to increased bond strength. In contrast, the absorption bands of amide C = O and phosphate P-O bonds were shifted to lower wavenumbers by ca. 1.04 and 9.53 cm^{-1}, respectively, indicating the bond weakening. The change in wavenumber of stretching vibrations of the functional groups displayed that these functional groups can be responsible for the effective performance of bacteria during Pb(II) uptake. Accordingly, we could argue that the Pb(II) ions are bound to the functional groups of *Bacillus amyloliquefaciens* ON261680.1 cell surface through complexation mechanism by providing overall negative charge of the bacterial cell surface along with selective chelating agents for binding to this toxic Pb^{2+} metal cations.

The information about attribution of Pb bio-precipitation and bio-adsorption by microbial metabolism is relatively rare (Zhu et al., 2019). XRD of the treated bacterial cell surface (Fig. 8) showed strong peak at 30.287o, corresponding to pyromorphite ($Pb_5(PO_4)_3Cl$ (Log *Ksp* = − 84.4)) (Zhang et al., 2020) as indicated by comparing the XRD spectra in Fig. 8**b** with the standard card (JCPDS card: 00–019-0701) provided by the International Centre for Diffraction Data using X Pert High Score Plus. The supernatants contained many components from the culture and metabolites of *Bacillus amyloliquefaciens* ON261680.1, such as PO_4^{2-}, Cl^- and low molecular weight organic acids, which could react with Pb^{2+} for the formation of the corresponding insoluble complex. Furthermore, the significance of pH factor observed in the optimization of *Bacillus amyloliquefaciens* ON261680.1 indi-

Table 5 FTIR spectral characteristics of the cell surface of *Bacillus amyloliquefaciens* ON261680.1 before and after Pb(II) ions adsorption.

Functional Groups of IR absorption bands	Wavenumber cm^{-1}		
	Before adsorption of Pb(II) ion	After adsorption of Pb(II) ion	Shift differences
	Absorption bands		
O–H	3439.65	3437.29	2.36
N–H			
C = O (amide)	1638.81	1639.85	1.04
P-O	1049.67	1059.20	9.53

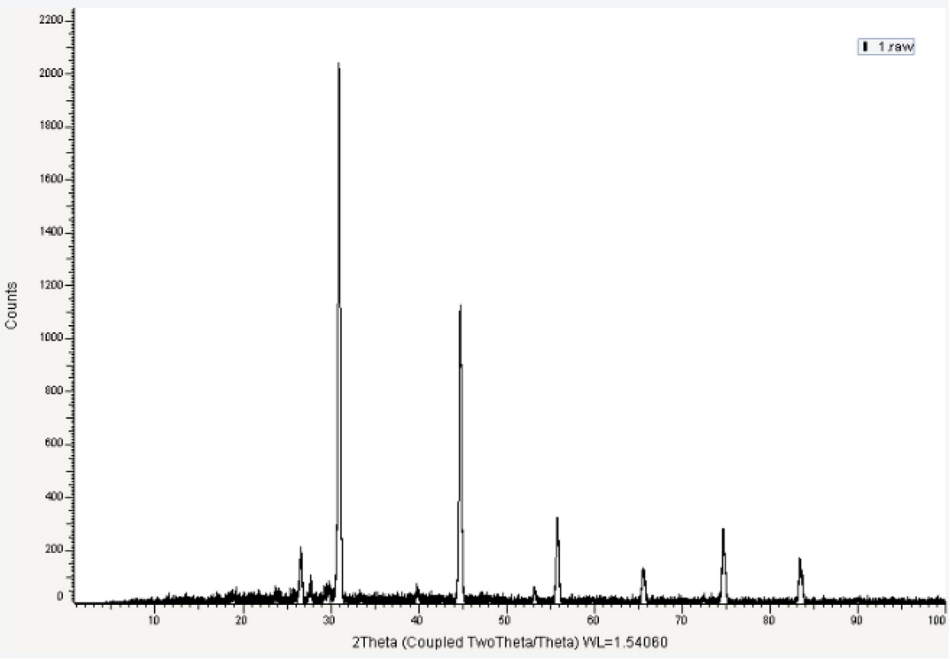

Fig. 8 XRD analysis of Pb^{2+} treated cells.

cates that ion exchange is also involved in lead removal (Fig. 9).

This was in agreement with that reported by Jaafar et al. (2016) who declared the possible mechanism of Pb bioremediation using *Deinococcus radiodurans* is through Pb (II) immobilization on microbial cell surface.

Biomineralization of heavy metals through biological treatment, especially microbial bioremediation was recently regarded as an efficient and attractive remediation technique and has been widely employed *in situ* (Chen et al., 2016). Several studies have proposed a significant mechanism of biomineralization associated with the formation of pyromorphite (Bai

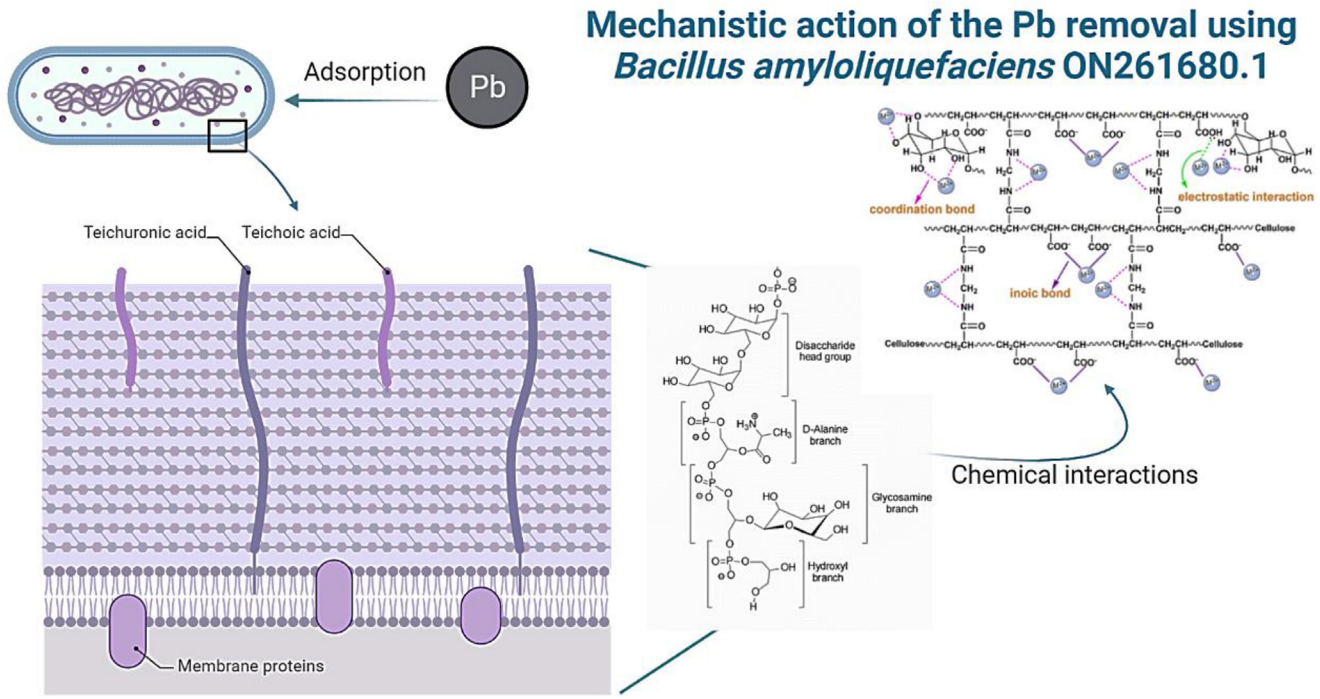

Fig. 9 Mechanism of Lead adsorption in *Bacillus amyloliquefaciens* ON261680.1 cells.

et al., 2014) which is a stable insoluble complex that hindering Pb bioavailability and toxicity in the environment (Mohapatra et al., 2019). Moreover, pyromorphite formation is suggested as a remediation management for Pb immobilization (Meng et al., 2020).

4. Conclusion

Data of the present study reported that *Bacillus amyloliquefaciens* ON261680.1 can be considered as a novel lead- resistant bacterium isolated from industrial effluents. The results concluded that Taguchi approach was an effective statistical design in optimizing the environmental and nutritional factors for the maximum bioremediation of lead by *Bacillus amyloliquefaciens* ON261680.1 reaching 99.84% removal. Furthermore, several mechanistic action studies showed that lead was deposited and precipitated on *Bacillus amyloliquefaciens* ON261680.1 cell surface through EDX, XRD, FTIR and TEM studies. Data confirmed the evolvement of the cell wall functional groups in Pb bio-adsorption on the bacterial cell surface in a form of pyromorphite ($Pb_5(PO_4)_3Cl$ through complexation mechanism by providing overall negative charge to the cell surface along with selective chelating agents for binding to this toxic Pb^{2+} metal cations. In general, the present study indicates the effectiveness of *Bacillus amyloliquefaciens* ON261680.1 in lead bioremediation to adjuvant pyromorphite (insoluble and less toxic complex) which can pave the way for biological treatment of industrial wastewater and polluted areas.

5. Financial & competing interests' disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Declaration of Competing Interest

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

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