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ORIGINAL RESEARCH ARTICLE

Biomass Assessment and Optimization of *Alcaligenes faecalis* Isolated from some Nigerian Mining Sites for Heavy Metal Uptake Using Response Surface Methodology Model.

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ABSTRACT

In this study, we investigated the heavy metal absorption of *Alcaligenes faecalis* strain U.B.I., a bacteria isolated from a mining site, under different environmental conditions. We utilized both conventional and molecular techniques to identify the bacteria and employed response surface methodology (R.S.M.) to determine optimal environmental conditions for heavy metal absorption. Our analysis revealed that the heavy metal-tolerant bacteria belong to the Proteobacteria, specifically the Betaproteobacteria order in the Burkholderiales family. Additionally, the bacteria's phylogenetic characteristics indicated a close relationship between the *Aeromonas* sp. cluster and members of the Aeromonadaceae family. Our results showed that the biomass *A. faecalis* strain U.B.I. had an optimal potential for chromium (Cr^{+}) absorption at 93.0%. We also conducted tests on the biomass under optimized conditions for lead (Pb^{2+}) absorption using R.S.M., resulting in a mean heavy metal uptake of 89.99%. Furthermore, we analyzed the surface functional groups after interaction with heavy metals and observed a significant shift in position of the functional groups. The O-H stretch and H-bonded at the 3268 cm^{1} position, while C=C stretch and N-O asymmetrical stretch/C-O stretch occurred at positions 2195 cm^{-1} and 1629 cm^{-1} of the spectra, respectively. Our findings suggest that the biomass of *A. faecalis* strain U.B.I. has potential for heavy metal bioremediation and can be used for heavy metal biosorption under various environmental conditions.

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Alcaligenes, Biomass, Biosorption, Heavy Metals, Optimization, Response Surface Methodology.

INTRODUCTION

Due to human activities, environmental contaminants have been released widely, leading to the pollution of soil, water, and air environments (Malik *et al.*, 2023; Huda *et al.*, 2023). Among these contaminants are heavy metals, considered some of the most ubiquitous pollutants affecting both the environment and biota. Recent research has linked environmental contamination by these heavy metals to rising ecological and global public health risks (Chen *et al.*, 2022). Human exposure has also increased significantly due to the exponential surge in their use in various industrial, agricultural, household, and technical applications. Several sources, including geogenic, industrial, agricultural, pharmaceutical, domestic effluent, and atmospheric sources, have reportedly contributed to the presence of heavy metals in the environment. Mining, foundries, smelters, and other metal-based industrial processes serve as significant point sources of heavy metal pollution (Alsafran *et al.*, 2022). Although heavy metals can biologically change into less harmful forms, their contamination remains a serious hazard to human life and a major global problem (Priya *et al.*, 2022). High concentrations of heavy metals can damage plant metabolism, affecting both the quality and quantity of food produced (Alsherif *et al.*, 2022). Heavy metals are also

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considered powerful mutagens and carcinogens that impact human health and well-being (Saravanan *et al.*, 2021).

Heavy metals are regarded as priority pollutants by the United States Environmental Protection Agency (USEPA), with lead (Pb) being the most dangerous element, followed by mercury (Hg), arsenic (As), and cadmium (Cd) as the sixth most poisonous metal, according to the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) (Yuan and Wang, 2022). A major concern is the high concentration of heavy metals in terrestrial and aquatic environments, acting as ecological toxins (Budianta, 2021). Industrial discharge, automobiles, and roads are the main causes of heavy metal pollution as the emissions contain heavy metals such as Cd, Pb, and As (Nogueira *et al.*, 2013). Fields can become contaminated with sewage sludge, leading to the accumulation of heavy metals in the soil and on plants. Each year, millions of metric tons of heavy metals, including 1 million metric tons of nickel (Ni) and 5 million metric tons of lead (Pb), are discharged into the soil (Onat *et al.*, 2013; Jaspal *et al.*, 2023; Fan *et al.*, 2023). Similarly, leachates from solid waste disposal, mining, and industrial waste directly contaminate groundwater with various toxic elements (Essien *et al.*, 2022). A significant problem is the transport of hazardous metals through the food chain. Research indicates that heavy metals with no known biological function, such as Cd and As, are hazardous even at low concentrations (Bharti and Sharma, 2022), while heavy metals that operate as co-factors may be necessary in small quantities but become poisonous at higher doses (Lucia *et al.*, 2023). Some heavy metals, like zinc (Zn), iron (Fe), copper (Cu), cobalt (Co), and molybdenum (Mo), are needed by humans in trace amounts but can be harmful in higher concentrations (Balali-Mood *et al.*, 2021). Toxic heavy metals like As, Pb, Cd, and Hg, which are not needed by the human body, can cause cancer if they accumulate over time (Balali-Mood *et al.*, 2021). The buildup of toxic metals in the body can impair the function of the kidney, bones, liver, heart, brain, and other organs, as they displace essential minerals, interfering with biological processes (Rai *et al.*, 2019).

In Nigeria, several studies have reported the impact of heavy metal pollution on soil (Ibrahim *et al.*, 2021), water (Nwazue *et al.*, 2022), air (Okoye and Ebiana, 2022), crops (Sagagi *et al.*, 2022), and farmlands (Sagagi *et al.*, 2022). Similarly, hazardous metals like Cr, Cu, Ni, Pb, and Co have been shown to contaminate most of Zambia's wastewater, crops, and soil (Kapungwe, 2013). When wastewater is used to irrigate crops in Egypt, there is an excessive buildup of heavy metals in the soil and plants (Nguyen *et al.*, 2018). Monitoring heavy metal pollution is a significant concern in Southeast Asian nations, including Bangladesh, Pakistan, India, Indonesia, and Thailand (Shaji *et al.*, 2021). According to the Central Pollution Control Board of India, Gujarat, Maharashtra, and Andhra Pradesh produce 80% of all hazardous waste in India, including toxic heavy metals (Ojha and Rahman, 2023). Although Cd is the most mobile metal and easily accessible to crops, several plants such as parsley (Petroselinum crispum), beet leaf (Beta vulgaris), coriander (Coriandrum sativum), radish leaf (Raphanus sativus), and basil (Ocimum basilicum) have been found to contain toxic heavy metals like Zn, Pb, Cd, As, and Cr in northeastern Iran (Sodhi *et al.*, 2022). Industrial effluents damaging water quality and potentially affecting soil quality, combined with rapid modernization and industrialization, have negatively impacted air, soil, and water quality. Dissolved hazardous metals are released into water bodies through mineral processing, electroplating, and paint formulation, leading to an increased concentration of harmful metals in the water (Samanta *et al.*, 2017). The consumption of heavy metals through drinking water can cause skin illnesses, respiratory, digestive, and renal problems (Munir *et al.*, 2022).

Heavy metal pollution is a significant environmental problem with serious health and ecological consequences. Addressing heavy metal pollution requires a combination of preventive measures, including the implementation of cleaner industrial techniques (Priya *et al.*, 2023) and the use of sustainable remediation methods such as phytoremediation (Singh and Pant, 2023), bioremediation, soil amendments (Demarco *et al.*, 2023), and other regulatory actions, such as transitioning to cleaner energy sources that can reduce the environmental impacts of mining and burning fossil fuels, which release heavy metals. Some key problems associated with heavy metal pollution include its toxicity to humans and wildlife, leading to acute and chronic health problems, including neurological disorders (Azar and Vajargar, 2023), kidney damage (Smereczański and Brzóska, 2023), cancer (Parida and Patel, 2023), and developmental issues in children (Zheng *et al.*, 2023). Heavy metals can also negatively impact aquatic food chains, increasing the risk of toxicity for top predators (Li *et al.*, 2023) and disrupting ecosystems by harming key species, reducing biodiversity, and altering nutrient cycling (Li *et al.*, 2023). This can lead to the exposure of sensitive species, resulting in imbalances within ecosystems (Angon *et al.*, 2023). Moreover, heavy metal contamination of soils can reduce soil fertility and impair plant growth, with negative implications for agriculture, as crops grown in contaminated soils may absorb heavy metals, posing a risk to food safety and security (Mbarki *et al.*, 2022). Heavy metals can leach from contaminated soils and enter groundwater, making it challenging to remediate and posing a risk to drinking water supplies (Xie *et al.*, 2023). Additionally, industrial processes and activities like mining, smelting, and combustion can release heavy metals into the atmosphere, leading to air pollution and potential respiratory health issues for nearby populations.

To address these problems, it is crucial to adopt effective pollution prevention measures, enforce regulations, and implement remediation strategies to reduce heavy metal pollution and its associated risks to human health and the environment. The use of bacterial biomass in this study to absorb heavy metals aims to assess the potential of a bio-based and cost-effective method for the removal of heavy

metal pollutants from environmental matrices. This approach, often referred to as bioremediation or biosorption, harnesses the unique abilities of certain species to accumulate heavy metals from their surroundings. The primary objective of using bacterial biomass to absorb heavy metals is to provide a sustainable, efficient, and environmentally friendly solution to mitigate the adverse effects of heavy metal pollution on both human health and the natural environment. The use of bacterial biomass may aid in assessing its potential for the remediation of contaminated sites and the restoration of ecosystems affected by heavy metal pollution.

METHODOLOGY

Characterization and Confirmation of Isolates using Conventional and Molecular Methods

The morphological (Grams reaction and spore staining) and biochemical characteristics (catalase, coagulase, sugar production, starch hydrolysis, etc.) of isolated bacteria from soils of a local mining site in Bagega District (11.8648°N, 6.0024°E) of Anka Local Government in North-west Nigeria were confirmed using conventional techniques. The bacterial isolates were further characterized using molecular techniques. The isolates were cultured as single colonies in nutrient broth at 37ºC for 24 hours. After harvesting cells from 5ml of broth and adding 100 µl of lysozyme for 30 minutes incubation, 700 µl of cell lysis buffer (comprising SDS, Tris-EDTA, etc.) was introduced. The vial was gently inverted for 5 minutes to mix the content, and DNA was subsequently precipitated from the aqueous layer using ethanol. The resulting DNA pellet was dried and then dissolved in 50 µl of 1x TE buffer. To assess DNA quality, it was examined using a 0.8% agarose gel stained with ethidium bromide (0.5 µg/µl). A single, concentrated DNA sample was used as a template for amplifying the 16s rRNA gene (as template DNA), following the method described by Zhang *et al.* (2000).

For the PCR reaction, universal primers were employed, with the forward primer having the sequence 5' AGAGTTTGATCMTGGCTCAG3' and the reverse primer with 5'TACGGYTACCTTGTTACGACTT 3'. A total of 25 µl of the PCR reaction solution was prepared, including 1.5 µl each of the forward and reverse primers, 5 µl of deionized water, and 12 µl of Taq master mix. The Taq master mix contained DNA polymerase, 2x tae buffer, 0.4Mm dNTPs, 3.2mM MgCl2, and 0.02% bromophenol blue. The PCR followed the following thermal cycling conditions:

- Denaturation: Initial heating of the DNA template at 94°C, breaking the hydrogen bonds and separating the DNA strands.
- Annealing: Cooling from 90°C to 60°C, allowing the primers to bind to complementary sequences in the DNA template.
- Extension: Heating to 72°C, the optimal temperature for DNA polymerase to extend the primers using the target DNA as a template (Zhang *et al.*, 2000).

The resulting DNA fragments were subjected to electrophoresis in agarose gels with a concentration of 1% and run in Tris-Acetic-EDTA (TAE) buffer (Bioline, UK). Ethidium bromide was used as a staining reagent. A loading buffer (containing bromophenol blue) was added to the samples, and in each gel, 3 µl of Ikb PCR molecular ladder (Bioline, UK) was loaded into the first well. Electrophoresis was conducted for 4 hours at 60 volts, and the reaction products were visualized using a gel documentation system (Alpha Innotech). The purified PCR product of the 16S rRNA gene from the bacterial isolate was submitted for sequencing using the ABI DNA 3730 XL sequencer (Applied Bio system). Sequencing was carried out in both directions, and the bacterial species were identified by comparing the obtained sequences with basic local alignment search tool (BLAST) searches. Following sequence matching and accession number acquisition, the sequences were submitted to the NCBI GenBank.

Biomass Production and Biosorption Experiment

After the bacteria were cultured in nutrient broth (N.B.) medium, their biomass was collected. A 72-hour culture was centrifuged for 15 minutes at 10,000 rpm to extract the biomass using a centrifuge (Selecta Centromix Model 220). After being cleaned twice with deionized water, the pellets were dried in the oven for 30 minutes at 100°C. Harvested biomass of identical volume was mixed for 24 hours on a rotary shaker at 160 rev/min in 1% nitric acid distilled water at a 12 ppm concentration for each heavy metal. After separating the biomass, the residual metal concentration in the supernatant was calculated using flame atomic absorption spectrophotometry (Garcia *et al.*, 2016). The amount of metal taken up by biomass is estimated as follows:

$$\text{Percentage uptake} = \frac{V(Ci - Cf)}{M} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where V= Volume of medium, Ci= initial concentration, Cf=final concentration, M=Mass of biosorbent

While the percentage uptake was determined thus;

$$\text{Percentage uptake} = \frac{q1 - q2}{q2} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: q1= quantity uptake before biosorption, q2= quantity uptake after biosorption.

Optimization of Parameters using Response Surface Methodology (R.S.M.); Model Design and Development

Two key aspects of the usage of R.S.M. were looked at: the performance of statistically prepared experiments and determining the coefficients in a mathematical model (Saravanan *et al.,* 2012).

Y = f (X1, X2, X3 .Xk) ………...………………(1)

Although the actual relationship between Y and Xk is typically unclear, equation 2 shows how a second-degree quadratic polynomial can describe the function in the relevant range (Uzun *et al.,* 2017).

$Z = \beta_O+\beta_1Y_1+\beta_2Y_2+\beta_3Y_3+\beta_4Y_4+ \beta_{11}Y_1+ \beta_{22}Y_{22}+ \beta_{33}Y_{33}+ \beta_{44}Y_{44}+ \beta_{12}Y_1Y_2+ \beta_{23}Y_2Y_3+ \beta_{34}Y_3Y_4+ \beta_{31}Y_3Y_1$…………………………………….....(2)

Where Z=Predicted value, βo=Constant, Y_1=Temperature (°C), Y_2=pH, Y_3=Inocula Size (mg/g), Y_4= Contact time (hours), β_1, β_2, β_3, β_4 are linear coefficients and β_{11}, β_{22}, β_{33} and β_{44} are quadratic coefficients. With pH (5 to 9), temperature (25 to 45°C), contact duration (24 - 72minutes), and inocula size (0.2-0.8mg/kg) as parameters, the low, middle, and high levels of each variable tested were marked -1, 0 and +1, respectively. In batch research, this also allows us to find significant interactions. These are approximated by the quadratic (second degree) polynomial (equation 2) where Y is the predicted value; βo is a constant; Y_1 is the inocula size (mg/g1); Y_2 is the pH, Y_3 is the contact time and Y_4 is the temperature (°C). Each variable's low and high levels of each variable are designated as -1, 0 and +1, respectively. Multiple linear regressions were utilized to determine the model's coefficients using a total of 30 runs.

Analysis of Surface Molecules Using FT-IR

Fourier transform infrared spectroscopy (FT-IR) the functional groups and chemistry of chemical bonding in the bacterial biomass were examined. Infrared spectra of the bacteria and biomass were obtained by mixing 200 mg of dry potassium bromide (KBr) powder and 200 mg of freeze-dried biomass in a mortar at a ratio of 1:100. The resultant slurry was compressed into transparent sample discs using a pressure bench press. The spectrometer (PerkinElmer Spectrum Version 10.4.3) was used to conduct the analysis, and spectrum data between 450 and 4000cm^{-1} was acquired and presented (Ramyakrishna, and Sudhamani, 2016).

Statistical Analysis

Descriptive statistics was used to analyze the data. Multiple regression analysis was also carried out to identify interaction by response surface using Design-Expert software (Stat Ease Incorporation Version 12).

RESULTS AND DISCUSSION

Morphological and Biochemical Characteristics of the Isolate

As presented in Table 1, the heavy metal-tolerant bacteria identified belonged to the phylum Proteobacteria, in the class Betaproteobacteria of the order Burkholderiales. Proteobacteria are a diverse group with various metabolic capabilities. This diversity allows them to adapt to different soil conditions and thrive in a wide range of environments. Some Proteobacteria can tolerate extreme conditions, such as high acidity or salinity, making them well-suited for diverse soil types (Zhang *et al.*, 2023). Proteobacteria play a crucial role in the decomposition of organic matter in soil. They are often involved in the breakdown of complex organic molecules such as dead plant and animal material, releasing nutrients like carbon, nitrogen, and phosphorus back into the soil (Yang *et al.*, 2023). To corroborate this study, Ivaldi *et al.* (2023) reported the isolation of abundant proteobacteria from soil extract. Similarly, Pham *et al.* (2023) reported the isolation of various groups of bacteria including proteobacteria from dioxin contaminated soil with biodegradation potential.

Table 1: Morphological, Biochemical and Molecular Characteristics of the Bacteria

Test	Result
Gram reaction	Negative
Motility	+
Cell Shape	Rod
Spore	-
Catalase	+
Lactose	-
Sucrose	-
Glu	-
Citrate	+
Indole	-
Methyl Red	+
Voges Proskauer	-
Nitrate Reduction	-
H_2S Production	-
Oxidase	+
Starch Hydrolysis	+
Accession Number	MT107249
Bacteria	*Alcaligenes faecalis* strain U.B.I.
Phylum	Proteobacteria
Genbank	National Center for Biotechnology Information

Molecular Identification of the Isolate

Results of the agarose gel electrophoresis carried out were shown in Figure 1. Additional phylogenetic characteristics of the bacteria revealed a close connection with the cluster of *Aeromonas* sp. and other members of the Aeromonadaceae family. Figure 2 depicts the relationship between *Alcaligenes faecalis* strain U.B.I. and other *Alcaligenes* species in the same group. The *Alcaligenes faecalis* strain U.B.I. identified in this research belonged to the class Betaproteobacteria and the Burkholderiales order of the Proteobacteria phylum. These species of bacteria employ several mechanisms that allow them to survive and thrive in environments contaminated with elevated levels of heavy metals (Abou-Aly *et al.*, 2019; Abou-Aly *et al.*, 2021). Development of efflux pumps, specialized membrane proteins that actively facilitate movement of metal ions out of cellular membrane prevents their accumulation to toxic level (Nguyen *et al.*, 2023). Because of their changing metabolic dynamics, this particular strain of bacteria was found to have significant tolerance to heavy metals in multiple investigations (Johnson *et al.,* 2019).

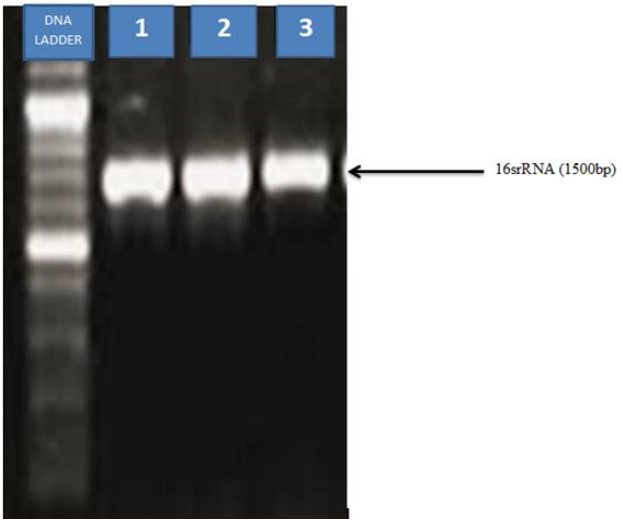

Figure 1: Agarose gel electrophoresis for PCR product of 16SrDNA showing the bacterial isolates labeled 1 (*Alcaligenes faecalis* strain UBI), 2 (*Aeromonassobria*), 3 (*Aeromonas* sp strain UBI)

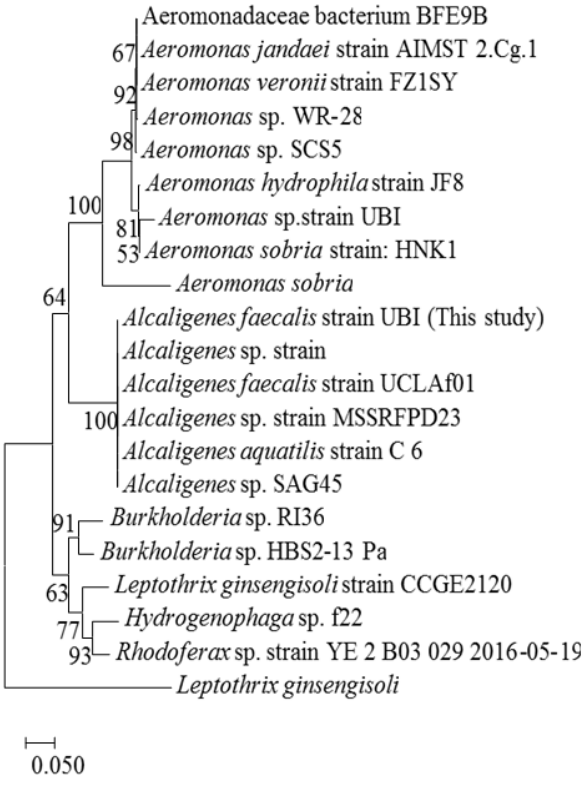

Figure 2: Phylogenetic tree based on 16SrRNA sequence using neighbor-joining method (Bootstrap values were ran at 1000 replications)

Biosorption Experiment

Figure 3 shows the results of an experiment in which bacteria were treated in a heavy metal-incorporated medium. According to the results, the highest uptake by the biomass was 93.0% of Cr^{+} with a biosorption rate of 93.0% (Cr > Cd > Pb > Cu). This means the biomass's potential for high uptake might result from its expanded surface-to-volume ratio. Studies by Tuzen *et al.* (2007), Srinath *et al.* (2002), and Benmalek and Fardeau (2016) demonstrated that dried bacterial biomass has a higher biosorption capacity than immobilized or living bacterial cells, with a capacity differential of up to 50% compared to their living isolates. Wrobel *et al.* (2023) further support this study's findings, who claimed that *Bacillus* sp. biomass absorbs more heavy metals from the environment than other corresponding bacterial cells.

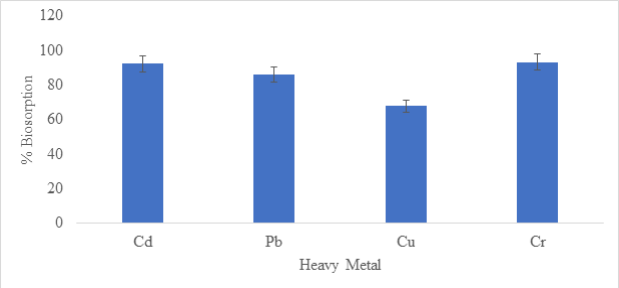

Figure 3: Percentage biosorption of heavy metals by Alcaligenes faecalis strain UBI biomass

Optimization of Heavy Metal Absorption using Response Surface Methodology (R.S.M)

The model for the respective biomass interaction with optimized variables is presented in Table 2. Similarly, interaction effect of variables on biosorption of Pb^{2+} by biomass is presented in Table 3. Multiple regression analysis of the observed responses resulted in the quadratic model thus: $Z = 80.23 - 7.05Y_1 + 0.725Y_2 + 0.120Y_0 - 1.19Y_4 - 0.206Y_{11} - 0.756Y_{22} + 0.718\ Y_{32} - 0.517Y_{42} + 0.43\ Y1Y_2 + 1.73\ Y_2Y_3 - 6.94\ Y_3Y_4 - 0.817Y_4Y_5$. Similarly, response surface interaction plots of different variables were presented in Figures 4 (pH and temperature), 5 (biosorbent dose and temperature), 6 (contact time and temperature), 7 (biosorbent dose and pH), 8 (contact time and pH), and 9 (biosorbent dose and contact time). The response surface methodology showed a R^2 value near 1, which indicates the model's accuracy with a non-significant lack of fit (0.601) (Yusuf *et al.*, 2016). Temperature was shown to substantially impact the biosorption process by the biomass, and the model developed was reliable ($p = 0.001$). According to Hlihor *et al.* (2014) and Adebanjo *et al.* (2022), temperature affects the biosorption capacity of biomass due to the thermodynamics and kinetic energy of aqueous interactions with metallic ions. In a study by Arasu *et al.* (2023), the main influence of temperature on the usage of *Bacillus* sp. biomass in an optimization process using R.S.M. was established. This study showed a statistically significant relationship between pH and temperature in Pb^{2+} biosorption (0.0088). The interaction of ions, chemical speciation, solubility, and charge of the biosorbent may simultaneously impact on these parameters together with the applied kinetic energy, as Kanamarlapudi *et al.* (2018) observed.

Analysis of Surface Molecules using FT-IR

After interacting with heavy metals, surface molecules and functional groups showed O-H stretch and H-bonded at position 3268 cm1, whereas C=C stretch and N-O asymmetrical stretch/C-O stretch/CH_2[x]alkyl-halide

appeared at positions 2195 cm^{-1} and 1629 cm^{-1} of the spectra, respectively. Upon interaction with heavy metals, a very significant shift in position was seen in the functional groups. Similar functional groups with O.H. stretch, C-N stretch, and N-H bond were seen in the biomass with obvious positional shifts (Table 4). The chemistry of the surface molecules of the bacterial biomass analyzed in this study showed peaks between 3200 cm^{-1} and 3400 cm^{-1}, indicative of hydroxyl (O.H.) and carboxylic (COOH) stretches. Heavy metal can bind to the functional groups present on bacterial cell surfaces (Ayele and Godeto, 2021). These functional groups are important components of various molecules like lipopolysaccharides, proteins and lipids. This observation was supported by similar findings by Cabuk *et al.* (2005) and Anna and Zofia (2014), who reported that Pb^{2+} was bound to hydroxyl and carboxyl groups and amide and sulphonamide bioligands. In another study by Qiao *et al.* (2019) on the bio-immobilization of lead by *Bacillus* biomass recovered from contaminated soil samples, these substances were discovered on the surface of the bacteria. Additionally, peaks at 1640 cm^{-1} measure the presence of amide, aromatic, and alkene functional groups, respectively, while those at 2000 cm^{-1} and 2200 cm^{-1} show a variable stretch of alkene present in the bacteria identified. According to Faghihzadeh *et al.* (2016), the existence of these surface molecules is a sign that structural proteins are present. It should be noted that after the heavy metal experiment, the wavelength of all bacterial surfaces noticeably altered between 3200 cm^{-1} and 3400 cm^{-1}. This can be as a result of the conformational alterations and and disruptions primarily due to modification of the structural integrity of the cellular proteins susceptible to the stressors (Aryal, 2021). In their study, Satapute *et al.* (2019) reported similar observation on some heavy metal resitant bacteria. Similarly, in agreement to this findings, Chai *et al.* (2021) reported similar observation in a review on conventional and novel materials towards heavy metal adsorption in wastewater treatment.

Table 2: Complete Composite Design Model of Response Surface Methodology for Biomass of *Alcaligenes faecalis* strain U.B.I. Showing Actual and Predicted Values of Pb^{2+} Biosorption

Run	Temperature (ºC)	pH	Biosorbent Dose (mg/g)	Contact Time (minutes)	Actual (%)	Predicted (%)	Error Rate
1	45	9	0.9	72	60.4	64.79	4.39
2	25	5	0.9	24	74.8	78.89	4.09
3	35	3	0.6	48	81.8	75.50	6.3
4	25	5	0.9	72	76.8	77.68	0.88
5	35	7	0.6	48	80.6	80.23	0.37
6	25	9	0.3	72	72.8	75.19	2.39
7	35	7	0.6	48	80.5	80.23	0.27
8	45	5	0.3	72	56.4	60.69	4.29
9	35	7	0.6	48	80.6	80.23	0.37
10	45	9	0.3	24	62.3	67.18	4.88
11	25	9	0.9	24	77.4	78.86	1.46
12	15	7	0.6	48	74.8	66.55	8.25
13	25	5	0.3	72	63.2	71.42	8.22
14	45	5	0.9	24	58.9	62.26	3.36
15	35	7	0.6	96	83.4	74.49	8.91
16	45	9	0.9	24	65.4	61.40	4.0
17	55	7	0.6	48	40.1	38.37	1.73
18	35	7	0.6	48	81.9	80.23	1.67
19	45	9	0.3	72	63.5	63.64	0.14
20	**25**	**9**	**0.3**	**24**	**79.2**	**81.60**	**2.4**
21	35	7	0	48	82.7	76.57	6.13
22	35	11	0.6	48	82.1	78.42	3.68
23	35	7	1.2	48	80.9	77.05	3.85
24	35	7	0.6	48	81.4	80.23	1.17
25	25	5	0.3	24	78.2	79.56	1.36
26	35	7	0.6	0	80.3	79.24	1.06
27	25	9	0.9	72	73.7	79.37	5.67
28	45	5	0.9	72	62.1	63.92	1.82
29	45	5	0.3	24	67.4	65.95	1.45
30	35	7	0.6	48	76.4	80.23	3.83

Table 3: ANOVA Model of R.S.M. Design for Biomass of *Alcaligenes faecalis* strain U.B.I.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Fit Statistics
Model	2635.43	14	188.25	5.61	0.0010*	-
A	1191.45	1	1191.45	35.52	0.0001*	-
B	12.76	1	12.76	0.3805	0.5466**	-
C	0.3504	1	0.3504	0.0104	0.9199**	-
D	33.84	1	33.84	1.01	0.3310**	-
AB	0.6806	1	0.6806	0.0203	0.0088*	-
AC	9.15	1	9.15	0.2728	0.6091**	-
AD	8.27	1	8.27	0.2465	0.6268**	-
BC	4.31	1	4.31	0.1284	0.7251**	-
BD	2.98	1	2.98	0.0887	0.7699**	-
CD	47.96	1	47.96	1.43	0.2503**	-
A^2	1322.09	1	1322.09	39.42	< 0.0001*	-
B^2	18.34	1	18.34	0.5468	0.4710**	-
C^2	20.06	1	20.06	0.5981	0.4513**	-
D^2	19.48	1	19.48	0.5808	0.4578**	-
Residual	503.08	15	33.54			-
Lack of Fit	483.90	10	48.39	58.62	0.601**	-
Std. Dev.	5.79	-	-	-	-	-
Mean	-	-	-	-	-	72.67
C.V.	-	-	-	-	-	4.97
R^2	-	-	-	-	-	0.9397
Adjusted R^2	-	-	-	-	-	0.9014
Predicted R^2	-	-	-	-	-	0.9031
Adequacy Precision	-	-	-	-	-	110.5575

Key: A: Temperature; B: pH; C: Biosorbent dose; D: Contact time; *:Significant; **: Not significant

Table 4: Surface chemistry of biomass obtained by FTIR spectrocopy

Before	After	Surface Molecules
3272.76	3272.80	OH stretch, N.H. stretch, CH stretch
2918.77	2950.84	O-H stretch
2996.82	2918.79	Medium CH Stretching
1629.54	2851.84	N-O asymmetric stretch,
1529.54	1629.56	N-O stretch,
1402.72	1529.60	N-O Stretching
1230.72	1462.77	S=O stretching
1313.78	1313.80	C-N Stretching
1056.77	1231.77	S=O stretching, CO stretching

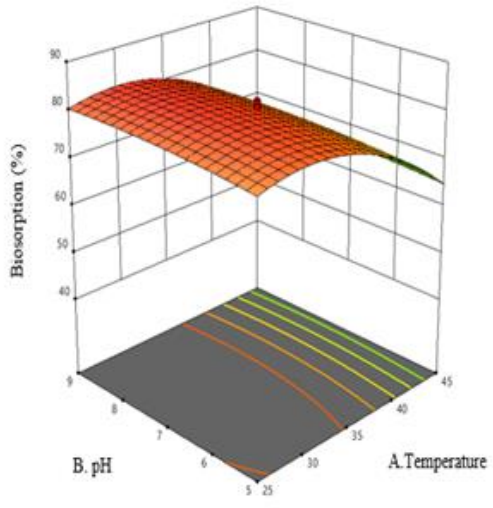

Figure 4: Graphical representation of response surface interaction in biomass of Alcaligenes faecalis strain U.B.I. between (B) pH and (A) temperature on percentage biosorption of Pb^{2+}

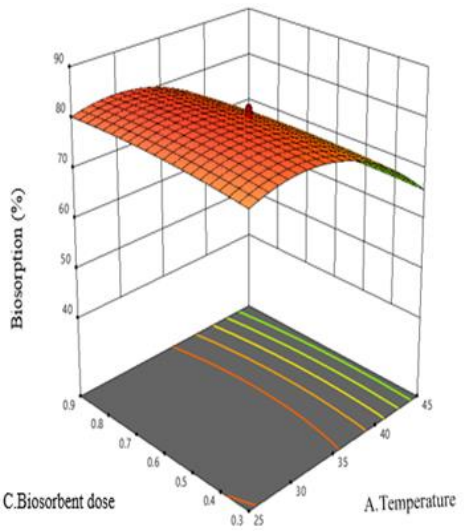

Figure 5: Graphical representation response of surface interaction in biomass of Alcaligenes faecalis strain U.B.I. between (C) biosorbent dose and (A) temperature on percentage biosorption of Pb^{2+}

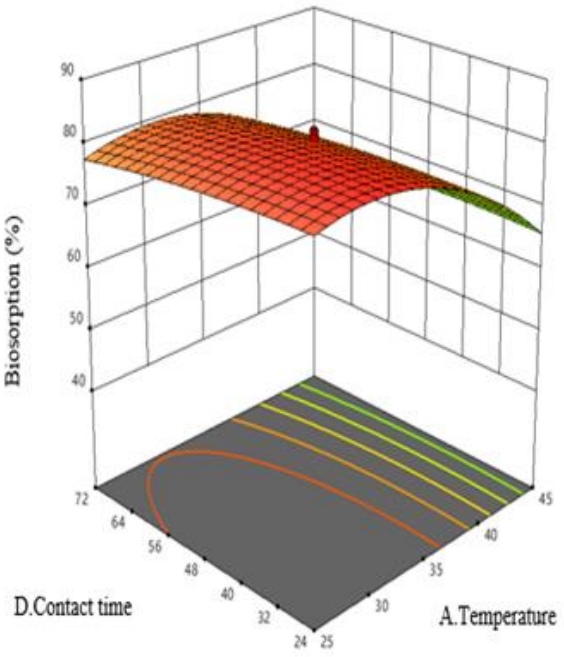

Figure 6: Graphical representation of response surface interaction in biomass of Alcaligenes faecalis strain U.B.I. between (D) contact time and (A) temperature on percentage biosorption of Pb^{2+}

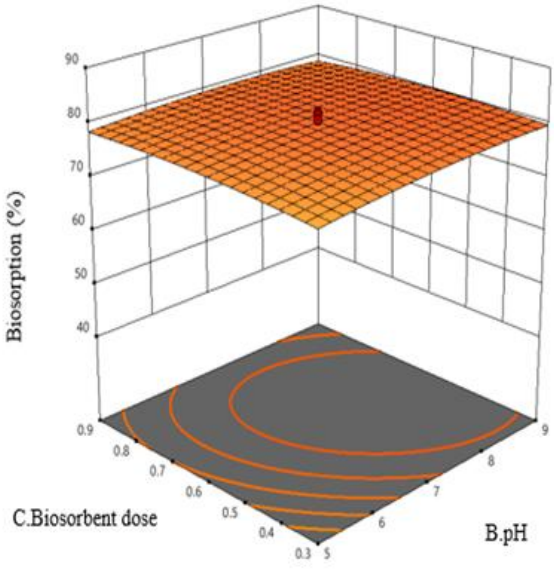

Figure 7: Graphical representation of response surface interaction in biomass of Alcaligenes faecalis strain U.B.I. between (C) biosorbent dose and (B) pH on percentage biosorption of Pb^{2+}

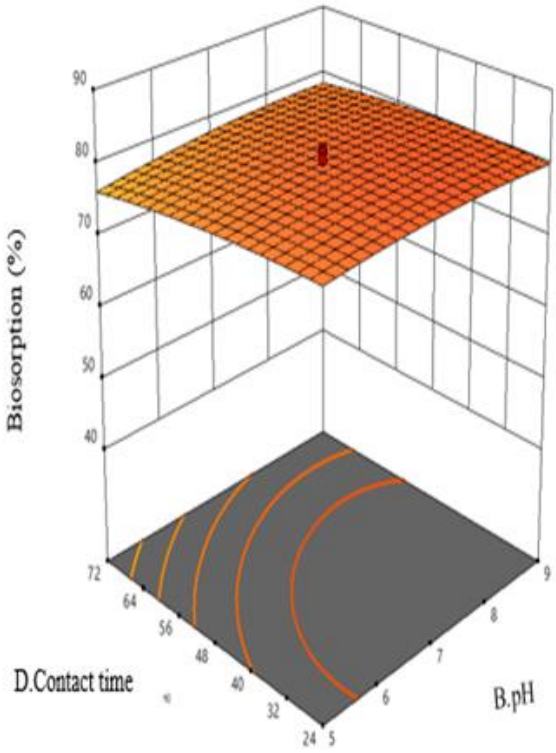

Figure 8: Graphical representation of response surface interaction in biomass in Alcaligenes faecalis strain U.B.I. between (D) contact time and (B) pH on percentage biosorption of Pb^{2+}

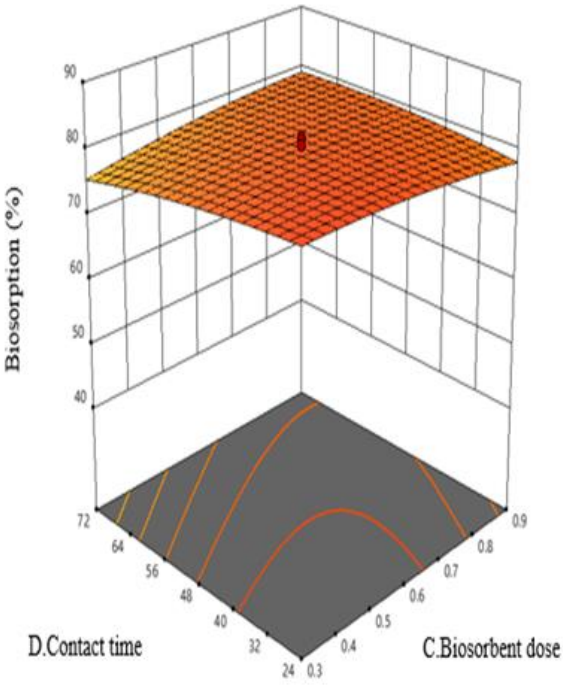

Figure 9: Graphical representation of response surface interaction in biomass of Alcaligenes faecalis strain U.B.I. between (C) biosorbent dose and (D) contact time on percentage biosorption of Pb^{2+}

CONCLUSIONS

In conclusion, the biomass of *Alcaligenes faecalis* strain U.B.I. utilized in this study belonged to a group of bacteria that was stated to have a high tolerance for heavy metals, and its biomass can be employed as a biosorbent to remove heavy metals.

RECOMMENDATIONS FOR FUTURE RESEARCH

It is recommended that;

i. Advanced analytical techniques (e.g., ICP-MS) should be employed in further studies to quantify the amount of heavy metals accumulated by the bacterial biomass.

ii. The dynamics of bacterial biomass in biofilm formation should be studied to further understand the organization and interaction in the community.

COMPETING INTERESTS

There is no competing interest to declare relevant to this article's content.

AUTHORS' CONTRIBUTIONS

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AVAILABILITY OF DATA

All sequencing data is available at NCBI GenBank https://www.ncbi.nlm.nih.gov/nuccore/MT107249.1/.

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