

Cd(II) biosorption using bacterial isolates from sawdust: optimization via orthogonal array Taguchi method

Lekan Taofeek Popoola¹, Adeyinka Sikiru Yusuf¹, Babatunde Kazeem Adeoye² and Tajudeen Adejare Aderibigbe³

¹Unit Operation and Material Science Laboratory, Chemical and Petroleum Engineering Department, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

²Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria

³Science Laboratory Technology Department, Yaba College of Technology, Yaba, Lagos State, Nigeria

Orthogonal array of Taguchi experimental design with L_{16} four-level factors: pH (2–8), temperature (303–333 K), time (1–4 h), inoculum concentration (5–20 v/v) and Cd(II) initial concentration (50–200 mg/L) was applied to optimize Cd(II) biosorption from aqueous solution via bacterial isolates from sawdust. The optimum conditions were found to be 4, 303 K, 4 h, 15 v/v % and 50 mg/L for pH, temperature, time, inoculum concentration and Cd(II) initial concentration, respectively. A confirmatory experimental run at these conditions revealed 99.53% Cd(II) removal. Fourier transform infrared revealed the presence of –OH on the bacterial surface enhancing Cd(II) biosorption. The presence of small cavities on the bacterial surface with a porous inner multilayer was shown by scanning electron microscopy analysis. Proposed biosorption mechanisms were electrostatic interaction, surface complexation and ion exchange. In conclusion, bacterial isolates from sawdust could effectively be applied as biosorbent for Cd(II) removal from aqueous solution.

INTRODUCTION

Water is among the natural resources needed for germination of crops, to achieve sustainable food production via irrigation (Hristov, 2014). Various forms of anthropogenic activities contaminate this natural resource when humans engage in industrial activities to manufacture end-products for consumption (Abdulla et al., 2019). This adversely affects the water and makes it unsuitable for agricultural purposes (fishing and irrigation). Sewage sludge and industrial effluents discharged into water bodies contain contaminants. Among these are heavy metals such as cadmium, lead, chromium, mercury and arsenic, whose presence at low concentrations stunts plant growth by altering its biochemical and physiological development, leading to chlorosis, root growth inhibition and plasma membrane damage (Bouazizi et al., 2010; Mkadmi et al., 2018; Nagendrappa et al., 2010). Declining plant growth decreases yield, which affects food supplies. Remediation of heavy metal contaminated water is thus important for food security.

In the past, physical and chemical methods (electrokinetics, solidification, soil washing, vapour extraction, encapsulation, stabilization, vitrification and so on) employed in tackling heavy metal-contaminated water have been prohibitively expensive (Popoola et al., 2018). They were not environmentally friendly and also altered the physico-chemical properties of water, thus making it unsuitable for agricultural purposes (Marques et al., 2009). Currently, researchers are focusing on applying biological techniques (bioremediation) to treat heavy metal-polluted water, because of the many advantages it offers over the previously mentioned methods (Patrón-Prado et al., 2010; Jacob et al., 2018). The most prevalent cost-effective and reliable bioremediation method is the application of microorganisms (bacteria, fungi, algae and yeast) isolated from plants to remove contaminants from polluted water (Kumar et al., 2010). Their mechanisms of heavy metal biosorption include transport across the cell membrane, complexation, ion exchange, precipitation and physical adsorption (Javanbakht et al., 2014). Studies have shown cadmium to be the highest ranked heavy metal in terms of damage caused to plant growth and human health (Ding et al., 2012; Hu et al., 2012). *Saccharomyces cerevisiae* (Hamza et al., 2010), dried seaweed *Sargassum sinicola* (Patrón-Prado et al. 2010), *Spirulina* sp. (Chojnacka et al., 2005), *Pseudomonas plecoglossicida* (Guo et al. 2012), *Tetraselmis suecica* (Pérez-Rama et al., 2010), NaOH-treated *Mucor rouxii* (Yan et al., 2003), *Pseudomonas fluorescens* (Sankarammal et al., 2014), *Klebsiella planticola* (Sharma et al., 2000), *Kocuria rhizophila* (Haq et al., 2015), *Cystoseira barbata* (Yalçın et al., 2012), dry biofilms from biotrickling filters (He et al., 2018), wheat straw (Wu et al., 2019), *Musa acuminata*–*Solanum tuberosum* peels (Rehman et al., 2019), microwave-assisted thiourea-modified *Sorghum bicolor* agrowaste (Salman et al., 2020), *Licheniformis* sp. and *Laterosporus* B. (Zouboulis et al., 2004) have been used for Cd(II) biosorption from polluted water.

Previously, two-level Plackett–Burman factorial design was used as an optimization tool for removal of Pb^{2+} using *Gelidium amansii* (El-Naggar et al., 2018a), and Cr^{5+} and Cu^{2+} using *Aspergillus terreus* (Abou-Taleb et al., 2017), while response surface methodology has been applied for removal of phenol using *Pseudomonas putida* (Sridevi et al., 2011) and of Pb^{2+} using *Aspergillus niger* (Amini et al., 2008) from aqueous solutions. However, application of Taguchi experimental design in different areas of wastewater treatment have gained serious attention over other experimental design methods due to

CORRESPONDENCE

Lekan Taofeek Popoola

EMAIL

ltpopoola@abuad.edu.ng

DATES

Received: 28 January 2020

Accepted: 15 September 2020

KEYWORDS

cadmium
bacterial isolates
biosorption
aqueous solution
sawdust

COPYRIGHT

© The Author(s)
Published under a Creative
Commons Attribution 4.0
International Licence
(CC BY 4.0)

its improved quality, and robust design (Daneshvar et al., 2007; Mousav et al., 2007; Barrado et al., 1996; Engin et al., 2008). Also, studies have shown the high resistance of *Pseudomonas aeruginosa* to pollutants including heavy metals, antibiotics, organic solvents and detergents (Haritash et al., 2009; Chellaiah, 2018).

This study applied L_{16} four-level factors (4^5) orthogonal array of Taguchi experimental design for investigating optimization of Cd(II) biosorption from polluted water using bacteria isolates from waste sawdust. The analysis of mean (ANOM) approach was adopted for statistical optimization of process parameters (pH, temperature, time, inoculum concentration and Cd(II) salt initial concentration) that influence Cd(II) biosorption from aqueous solution. Analysis of variance (ANOVA) technique was applied to check the percentage contribution of individual process parameters to the biosorption process. An experiment was conducted to correlate the predicted optimized condition by the Taguchi experimental design. Few investigations have been conducted in the past on application of bacteria isolates from sawdust as active bio-agents for the removal of Cd^{2+} from aqueous solution. Characterization was done using FTIR and SEM.

MATERIALS AND METHODS

Waste sawdust collection

A sawdust sample was collected from New Garage Saw Milling Industry, Ibadan, Oyo State, Nigeria. Dirt was removed from the sawdust by hand picking. It was then passed through a mesh size of 1.18 mm to obtain a smaller particle size. The sawdust was then washed thoroughly in distilled water and sieved. About 20 kg of sieved particle was later soaked in a bucket containing water at room temperature for 14 days to activate bacterial growth.

Analytical chemicals

Broth medium, nutrient agar, sodium hydroxide (NaOH), hydrochloric acid and trihydrates of cadmium nitrate ($Cd(NO_3)_2 \cdot 3H_2O$) were supplied by TopJ Scientific, Ajilusun Road, Ado-Ekiti, Nigeria. All the chemicals used were of analytical grade.

Isolation and characterization of bacteria

The dilution plate method was adopted to isolate and characterize bacterial isolates from the fermented sawdust. Ten grams of fermented sawdust was added to 90 mL distilled water. Twenty-three grams of nutrient agar (Lifesave biotech, USA) (peptic digest of animal tissue = 5.0 g/L, beef extract = 1.50 g/L, yeast extract = 1.50 g/L, sodium chloride = 5.0 g/L, agar = 15.0 g/L and final pH at $25^\circ C = 7.4 \pm 0.2$) were dissolved in 1 000 mL of distilled water and continuously stirred until homogeneity was ascertained. Ten different samples containing 9 mL of diluted sawdust and 10 mL of nutrient agar each were set up under aerobic conditions in a petri dish and placed in an incubator at $37^\circ C$ for 5 days. The discrete bacterial colonies were sub-cultured and sterilized. Stock cultures were then prepared from the pure cultures and stored at $4^\circ C$ for further laboratory work.

Bacterial isolates were characterized using the sugar fermentation test. The method described by Cheesbrough (1985) was adopted to conduct a motility test. The methods described by MacFadden (2000) were used to perform biochemical tests. The various tests executed include motility, methyl-red, catalase, glucose, sucrose, mannose, lactose, pigment, oxidase, gram reaction and citrate.

Batch biosorption experiments

Preparation of Cd(II) solution

One gram of 1 M $Cd(NO_3)_2 \cdot 3H_2O$ salt was dissolved in 1 L of distilled water to form simulated stock solutions in a 1 000 mL

round bottom flask. Different initial concentrations of Cd(II) solution were prepared in 200 mL conical flasks for batch biosorption process using a Taguchi experimental design, as shown in Table 1.

Batch biosorption process

A temperature-controlled stirrer (Stuart heat-stirrer SB162) was used to investigate the batch biosorption of Cd(II) from solution using 5-day cultured bacterial isolates. The values for pH, temperature, time, inoculum concentration and Cd(II) salt initial concentration investigated for the batch process were based on the values specified by L_{16} four-level factors (4^5) orthogonal array of Taguchi experimental design (Ghani et al., 2013) as presented in Table 2 (16 experimental runs). The pH of the solution was varied using 1 M HCl and 1 M KOH aqueous solutions. Constant values of 100 mL of Cd(II) salt in 250-mL flasks and 130 r/min were used throughout the experiment. Whatman PTFE filter paper was used to separate the filtrate from the residue. Cd(II) concentration was measured using an atomic absorption spectrometer (AAS Buck Scientific 210 VGP, USA). The concentrations of Cd(II) in the solution after a specified time were measured at a dilution factor of 50 and average values were recorded. The potential of bacterial isolates from sawdust to remove Cd(II) from solution was determined by measuring the percentage of Cd(II) adsorbed using Eq. 1.

$$\% \text{ Cd(II) Sorption} = \frac{(C_o - C_t)}{C_o} \times 100\% \quad (1)$$

where C_o is the initial concentration of Cd(II) (mg/L) and C_t is the final concentration of Cd(II) (mg/L) after each experimental run.

Optimization studies using analysis of mean technique

In order to execute optimization studies for Cd(II) biosorption from aqueous solution using bacterial isolates from sawdust, 5 operation factors were examined such that each of them was set at 4 different levels, as presented in Table 1. The experimental conditions for optimum biosorption of Cd(II) were determined using L_{16} orthogonal array of Taguchi experimental design as presented in Table 2. Signal-to-noise (S/N) ratio was calculated using Eq. 2 (Pundir et al., 2018) for statistical analysis to maximize the process conditions for optimum biosorption.

$$\frac{S}{N} = -10 \log \left(\frac{1}{n} \sum_{i=1}^n \frac{1}{R_i^2} \right) \quad (2)$$

where n = number of replications for each experimental run and R_i = percentage Cd(II) removed from solution in replication experiment i executed under the same experimental conditions for each test run.

The average S/N ratio value of each process parameter at a particular level was calculated using Eq. 3:

$$(M)_{Level=i}^{Factor=F} = \frac{1}{n_{Fi}} \sum_{j=1}^{n_{Fi}} \left[\left(\frac{S}{N} \right)_{Level=i}^{Factor=F} \right]_j \quad (3)$$

where $(M)_{Level=i}^{Factor=F}$ = mean value of S/N ratio with factor F at level i ,

$\left[\left(\frac{S}{N} \right)_{Level=i}^{Factor=F} \right]_j$ = S/N ratio value with factor F at level i in its j^{th} appearance ($j=1, 2, 3$ and 4) and n_{Fi} = number of appearances of factor F in level i .

Table 1. Biosorption process parameters and levels using L_{16} Taguchi experimental design orthogonal array

Factor code	Parameter	Unit	Level 1 (L1)	Level 2 (L2)	Level 3 (L3)	Level 4 (L4)
A	pH	-	2	4	6	8
B	Temperature	K	303	313	323	333
C	Time	hr	1	2	3	4
D	Inoculum concentration	v/v (%)	5	10	15	20
E	Cd(II) initial concentration	mg/L	50	100	150	200

Table 2. Experimental runs

Run	pH (A)	Temperature (K) (B)	Time (h) (C)	Inoculum conc. (v/v %) (D)	Cd(II) initial conc. (mg/L) (E)
1	6	333	2	5	150
2	2	313	2	10	100
3	4	333	3	10	50
4	6	313	4	15	50
5	8	333	1	15	100
6	4	313	1	20	150
7	4	303	2	15	200
8	8	313	3	5	200
9	2	303	1	5	50
10	8	303	4	10	150
11	2	333	4	20	200
12	2	323	3	15	150
13	8	323	2	20	50
14	6	323	1	10	200
15	6	303	3	20	100
16	4	323	4	5	100

Determination of factor percentage contribution using analysis of variance

Analysis of variance (ANOVA) was employed to determine the percentage contribution of each process factor (P_F) to Cd(II) biosorption using Eq. 4:

$$\rho_F = \frac{SS_F - (DOF_F \times V_{Er})}{SS_T} \times 100 \quad (4)$$

where SS_F , DOF_F , V_{Er} and SS_T are factorial sum of squares, degrees of freedom of each factor, variance of error and total sum of squares expressed as Equations 5, 6, 7 and 8, respectively.

$$SS_F = \frac{mn}{L} \sum_{k=1}^L \left((\bar{R})_k^F - \bar{R}_T \right)^2 \quad (5)$$

$$DOF_F = L - 1 \quad (6)$$

$$V_{Er} = \frac{SS_T - \sum_{F=A}^D SS_F}{m(n-1)} \quad (7)$$

$$SS_T = \sum_{j=1}^m \left(\sum_{i=1}^n R_i^2 \right) - mn(\bar{R}_T)^2 \quad (8)$$

where

m = number of experiments executed

n = number of replications of each experiment

L = number of levels of each factor

$(\bar{R})_k^F$ = cumulative average of Cd(II) percentage removal with a certain factor F at k^{th} level (expressed as Eq. 9)

\bar{R}_T = cumulative average of Cd(II) removed from solution (expressed as Eq. 10).

$$(\bar{R})_k^F = \frac{1}{n_{Fk}} \sum_{j=1}^{n_{Fk}} \left[(\bar{A})_{level=k}^{Factor=F} \right] \quad (9)$$

where

n_{Fk} = number of factor F appearances at level k

$\left[(\bar{A})_{level=k}^{Factor=F} \right]_j$ = average Cd(II) percentage removal (\bar{A}) with a factor F at level k in its j^{th} appearance sequence ($j = 1, 2, 3, \dots, n_{Fk}$).

$$\bar{R}_T = \frac{\sum_{j=1}^m \left(\sum_{i=1}^n R_i \right)_j}{mn} \quad (10)$$

where R_i = percentage Cd(II) removed from solution in replication experiment i executed under the same experimental conditions for each test run.

Characterization of the bacterial cell surface

The active functional groups present on the surface of the bacterial isolates were investigated using Fourier-transform infrared spectroscopy (Nicolet iS10 FT-IR Spectrometer, USA) within a wavelength of 400–4 000 cm^{-1} . Beams of infrared were directed at the sample. The quantity and frequencies at which samples absorbed IR light were measured. The sample molecular identities were determined using the reference database.

The morphology of the bacteria was studied using a scanning electron microscope (SEM-JEOL-JSM 7600F) operated under high-vacuum evaporation at 5 000 \times , 15 kV. The sample was coated using a low-vacuum sputter platinum coating and then placed in a relative high-pressure chamber. The electron optical

column was differentially pumped to ensure that the vacuum was kept adequately low. Secondary electron signal amplification was provided by the high-pressure region around the sample which neutralizes the charge. Due to the field emission gun's (FEG) ability to produce high primary electron brightness, low-voltage SEM was used in the FEG-SEM.

RESULTS AND DISCUSSION

Calculation of average percentage Cd(II) removal and signal-to-noise ratio at factor levels

Equation 1 was used to determine the percentage of Cd(II) removed from aqueous solution (A_i) for each of the experimental tests, which were replicated for $i = 1, 2, 3$ and 4 for specified values

of pH (2, 4, 6 and 8), temperature (303, 313, 323 and 333 K), time (1, 2, 3 and 4 hrs), inoculum concentration (5, 10, 15 and 20 v/v %) and Cd(II) initial concentration (50, 100, 150 and 200 mg/L) as stated in Table 1. The results obtained for A_i , average of responses for the experimental runs (\bar{A}_i) and signal-to-noise ratio (calculated by using Eq. 2) (S/N) are presented in Table 3. Equation 3 was used to estimate the average S/N ratio values (presented in Table 4) for a certain factor at a certain level (M_{Level}^{Factor}), while Eq. 9 was used to calculate the average Cd(II) removed from aqueous solution (presented in Table 5) for a certain factor at a certain level (\bar{R}_{Level}^{Factor}). The average percentage Cd(II) removed from aqueous solution and respective S/N ratio at different levels of pH, temperature, time, inoculum concentration and Cd(II) initial concentration are presented as Figs 1–5, respectively.

Table 3. Percentages of Cd(II) removed from aqueous solution and S/N ratios

Test	Cd(II) removed (%)					S/N ratio
	Response 1 (A_1)	Response 2 (A_2)	Response 3 (A_3)	Response 4 (A_4)	Average response (\bar{A})	
1	87.17	91.36	95.68	88.5	90.68	39.13
2	88.5	80.5	88.25	83.9	85.29	38.60
3	94.42	81.51	85.36	86.48	86.94	38.75
4	97.07	89.1	93.28	86.81	91.57	39.21
5	95.5	88.27	98.77	92.61	93.79	39.42
6	82.91	84.42	87.02	73.33	81.92	38.21
7	77.32	81.06	99.34	86.92	86.16	38.59
8	92.7	93.23	92.65	98.84	94.36	39.49
9	81.81	87.29	83.87	81.03	83.50	38.42
10	83.76	95.46	94.25	94.52	92.00	39.24
11	91.29	77.71	85.36	94.28	87.16	38.73
12	89.63	96.93	76.3	93.5	89.09	38.89
13	98.93	75.32	78.62	96.77	87.41	38.64
14	88.01	97.64	94.08	91.6	92.83	39.34
15	91.72	89.07	89.15	74.8	86.19	38.62
16	80.51	93.37	91.2	90.01	88.77	38.92

Table 4. S/N ratio of responses at factor levels

Factor level	$\left[\left(\frac{S}{N} \right)_{Level}^{Factor} \right]_j$				$(M)_{Level}^{Factor}$
	$j = 1$	$j = 2$	$j = 3$	$j = 4$	
A/1	39.13	38.6	38.75	39.21	38.92
A/2	39.42	38.21	38.59	39.49	38.93
A/3	38.42	39.24	38.73	38.89	38.82
A/4	38.64	39.34	38.62	38.92	38.88
B/1	39.13	39.42	39.49	38.64	39.17
B/2	38.60	38.21	39.24	39.34	38.85
B/3	38.75	38.59	38.73	38.62	38.67
B/4	38.21	39.49	38.60	38.92	38.80
C/1	39.13	38.64	39.24	38.21	38.81
C/2	38.60	39.42	38.73	38.62	38.84
C/3	38.75	38.42	38.89	39.49	38.89
C/4	39.21	38.59	39.42	39.13	39.09
D/1	39.13	38.21	38.73	38.92	38.75
D/2	38.60	38.59	38.42	38.64	38.56
D/3	38.75	39.49	39.24	39.34	39.20
D/4	39.21	39.42	38.89	38.62	39.03
E/1	39.13	39.49	38.62	38.62	38.97
E/2	38.60	38.73	38.64	38.92	38.72
E/3	38.75	38.89	39.34	38.64	38.90
E/4	39.21	38.42	38.92	38.21	38.69

Table 5. Percentage removal of Cd(II) at factor levels

Factor level	$\left[(\bar{A})_{Level}^{Factor} \right]_j$				$\left[(\bar{R})_{Level}^{Factor} \right]$
	$j = 1$	$j = 2$	$j = 3$	$j = 4$	
A/1	89.59	93.20	82.27	92.09	89.29
A/2	78.28	88.25	89.84	93.20	87.39
A/3	92.59	79.59	80.69	94.62	86.87
A/4	90.79	97.98	90.79	93.37	93.23
B/1	89.59	92.59	92.59	90.79	91.39
B/2	93.20	88.25	92.95	79.59	88.50
B/3	82.27	89.84	93.37	88.25	88.43
B/4	92.09	79.59	89.59	82.27	85.88
C/1	89.59	80.69	88.25	89.84	87.09
C/2	93.20	78.28	89.84	97.98	89.83
C/3	94.62	88.25	97.98	89.25	92.53
C/4	93.20	93.37	89.59	94.62	92.70
D/1	89.59	88.25	80.69	93.37	87.97
D/2	93.20	89.84	94.62	90.79	92.11
D/3	82.27	97.98	92.59	97.98	92.70
D/4	92.09	89.59	97.98	94.62	93.57
E/1	94.62	93.37	94.62	90.79	93.35
E/2	82.27	97.98	92.59	93.37	91.55
E/3	82.27	78.28	92.95	90.79	86.07
E/4	92.09	88.25	80.69	79.59	85.15

Process parameter analysis

Solution pH

The effect of solution pH on percentage of Cd(II) removed from aqueous solution and signal-to-noise ratio was investigated (Fig. 1). It was observed that the percentage of Cd(II) removed from solution decreased from 89.29 to 86.87% when the solution pH was increased from 2 to 6. This could be attributed to inactivity and death of some cells (reduction in population) of the bacterial isolates due to the presence of strong acid (Yan et al., 2003). Also, functional groups on the outer surface of the bacteria become positively charged at lower pH, thereby creating repulsion between their surface and Cd(II) in solution which reduces heavy metal removal from solution (Yan et al. 2003). Previous studies have reported reduced heavy metal removal from solution using microorganisms at a lower pH of 3 (Kassab et al., 2006; Engin et al., 2008). However, the percentage of Cd(II) removed from aqueous solution drastically increased in the basic medium (pH = 8) (Marques et al., 2009), which gives a favourable environmental condition that enhances the bacterial growth and thus increased their efficiency to remove more Cd(II) from solution. Also, the existence of negative charges at the surface of the bacteria at the higher pH value of 8 enhanced the strong electrostatic force between the negatively charged surface and Cd(II) in solution. Nevertheless, the presence of potassium in the KOH used to adjust the solution pH might have provided a nutrient source for the bacterial isolates' growth (Atlas et al., 1973). A similar study revealed formation of hydroxyl complexes, starting from pH 7, resulting from electrostatic interaction (Kassab et al., 2006). Previous studies have also revealed similar results for removal of nickel (Aka et al., 2016) and lead (Kassab et al., 2006) from solution using bacterial isolates from sawdust.

Reaction temperature

The effect of temperature on the average percentage Cd(II) removed from solution (in the presence of bacterial isolates) and signal-to-noise ratio is shown in Fig. 2. The result revealed

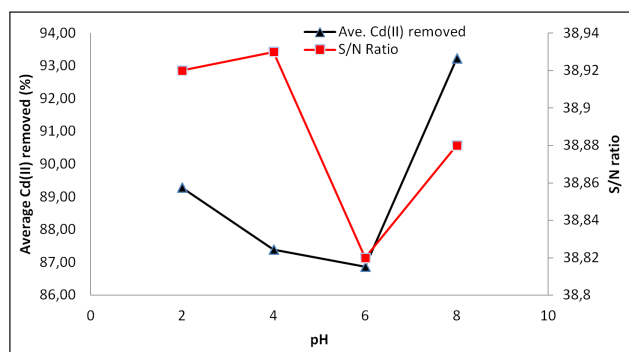


Figure 1. Solution pH against average Cd(II) removed and S/N ratio

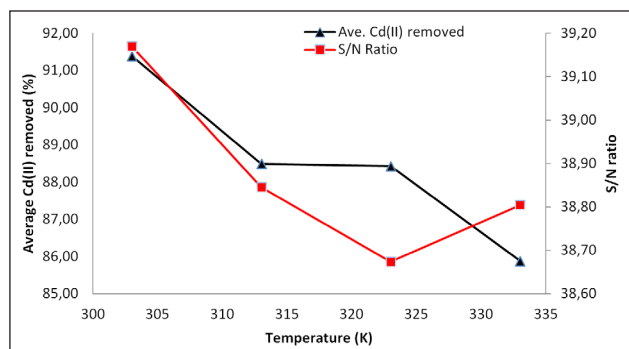


Figure 2. Solution temperature against average Cd(II) removed and S/N ratio

a decrease in the average percentage of Cd(II) removed from solution from 91.39 to 85.88% when the reaction temperature was increased from 303 to 333 K. This resulted from damage to proteins present in bacterial colonies, which in turn reduced their growth rate and metabolic activities at this higher temperature (Huang et al. 2016). Research has revealed that bacterial efficacy in removing heavy metals from solution varies from one to another under different temperature conditions (Kumar et al., 2010; Javanbakht et al., 2014). Some studies have produced similar results to the current study (Yan et al., 2003; Sankarammal et al., 2014).

Reaction time

Figure 3 presents the variation of average Cd(II) uptake from solution by bacterial isolates and signal-to-noise ratio at different levels of reaction time. As the reaction time increases, more of the Cd(II) was removed from solution by the bacteria as a result of an increase in their growth resulting from the formation of more colonies. However, partial equilibrium was attained after 3 h, substantiating the efficiency of the bacterial colonies in removing Cd(II) from aqueous solution as the percentage of heavy metal removed was almost constant. A high importance of reaction time in biosorption processes for heavy metals has been reported in several studies (Popoola, 2019a; Rao et al., 2006). Various equilibrium times attained for Cd(II) removal from solution have been reported, as 45 min (Haq et al., 2015), 90 min (Popoola, 2019b) and 120 min (Singh et al., 2000).

Inoculum concentration

A plot of inoculum concentration against average Cd(II) removed and S/N ratio is shown in Fig. 4. The average percentage Cd(II) removed from aqueous solution increased with an increase in the concentration of inoculums. A lower percentage removal of Cd(II) (87.97%) recorded at lower inoculum concentration (5 v/v %) resulted from lower populations of bacteria available to remove the heavy metal from solution. However, greater bacterial growth was observed at increased inoculum concentration (20 v/v %), which facilitated the biosorption of Cd(II) from solution.

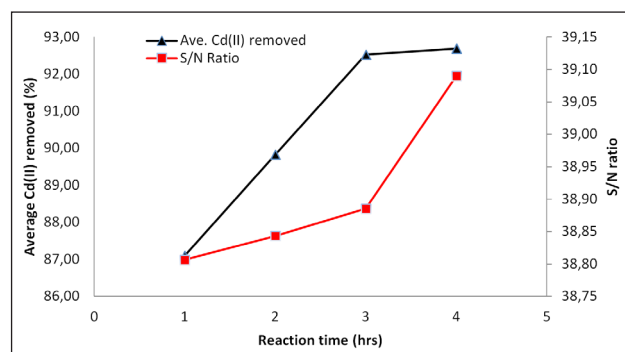


Figure 3. Reaction time against average Cd(II) removed and S/N ratio

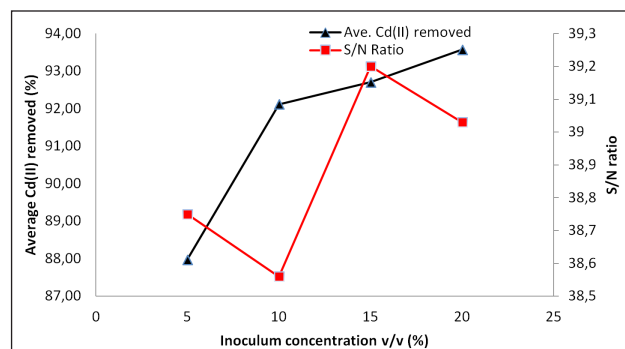


Figure 4. Inoculum concentration against average Cd(II) removed and S/N ratio

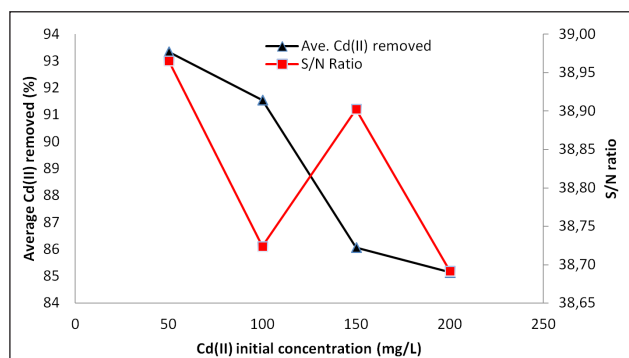


Figure 5. Cd(II) initial concentration against average Cd(II) removed and S/N ratio

At this point, more negatively charged bacteria having a greater total surface area were available at a constant pH of 8 (Dursun et al., 2003). The higher the concentration of biosorbent, the higher the efficiency of heavy metal removal from solution (Yaşın et al., 2012; Zouboulis et al., 2004).

Cd(II) initial concentration

Figure 5 presents a plot showing the effect of increased initial concentration of Cd(II) on the efficiency of bacterial isolates from sawdust in removing it from solution, and on the signal-to-noise ratio. It was observed that the percentage of Cd(II) removed from solution decreased slowly from 93.35 to 85.15% when Cd(II) initial concentration was increased from 50 mg/L to 200 mg/L. This observation could be attributed to: (i) reduction of binding sites (active agent of Cd(II) removal) on bacteria surface due to a reduction in their growth; (ii) inhibitory effect of cadmium salt on the bacterial growth which destroys the protein part of the bacteria; and (iii) presence of excess Cd(II) ions in solution which eventually reduced the accessibility of available binding sites to the heavy metal. Similar previous studies have also reported this observation for the removal of other heavy metals using microorganisms (Patrón-Prado et al., 2010; Kumar et al., 2010; Javanbakht et al., 2014; Hamza et al., 2010).

Predictive mathematical model development

In this study, the effect of 5 factors placed at 4 levels – pH (2, 4, 6 and 8), temperature (303, 313, 323 and 333 K), time (1, 2, 3 and 4 h), inoculum concentration (5, 10, 15 and 20 v/v %), Cd(II) initial concentration (50, 100, 150 and 200 mg/L) – was investigated using orthogonal arrays generated by design-expert (7.0.0). The experimental values obtained for the average percentage of Cd(II) removed from water using bacterial isolates from sawdust at different factor levels are presented in Figs 1–5. A general predictive model (developed using the Taguchi approach) which could be used to establish an empirical relationship between the average percentage of Cd²⁺ removed from water and independent process variables is stated as Eq. 11:

$$\begin{aligned} \text{Average Cd}^{2+} \text{ removed (\%)} = & +81.67 - 2.39 A[1] - 3.63 A[2] + \\ & 2.17 A[3] - 2.17 B[1] - 3.15 B[2] + 0.52 B[3] - 2.79 C[1] - \\ & 0.74 C[2] + 6.99 C[3] + 3.01 D[1] - 2.70 D[2] + 1.95 D[3] - \\ & 0.67 E[1] + 4.79 E[2] - 1.37 E[3] \end{aligned} \quad (11)$$

where A, B, C, D and E represent pH, temperature, time, inoculum concentration and Cd(II) initial concentration, respectively. The values indicated within the square brackets represent levels of the corresponding model terms.

Application of analysis of variance to evaluate factor percentage contribution

The value of \bar{R}_T was calculated using Eq. 10 to analyse the

percentage contribution of pH, temperature, reaction time, inoculum volume and Cd(II) initial concentration towards Cd(II) removal from aqueous solution by bacterial isolates. The calculated value of \bar{R}_T was 88.60. The factorial sum of squares, SS_F , for each factor (Table 6), was calculated via substitution of \bar{R}_T and \bar{R}_k^F into Eq. 5. The value of total sum of squares (SS_T) calculated via Eq. 8 was 535.85. The value of variance of error (V_{Er}) calculated by substituting SS_F and SS_T into Eq. 7 was 4.34. Thus, substitution of $DOF_F = 3$, SS_T and SS_F into Eq. 4 gives the percentage contribution of each factor (ρ_F) (Table 6) to Cd(II) removal from aqueous solution using bacterial isolates from sawdust.

The result obtained (Table 6) revealed the influence of each of the examined parameters (measured as a percentage) on Cd(II) biosorption from aqueous solution by bacterial isolates from sawdust. The order of influence was observed to be Cd(II) initial concentration (33.87%) > time (26.14%) > inoculum concentration (20.05%) > temperature (8.64%) > pH (6.07%). Studies have shown that the higher the initial concentration of heavy metal, the lower its removal from solution by adsorbents (Popoola, 2019b; Amini et al., 2008). Thus, initial concentration of a heavy metal greatly influences the rate of its biosorption from solution. This is because a high concentration of Cd(II) in solution hinders the growth of bacteria which adversely affects its efficiency in removing Cd(II) from solution. Hence, this affirms that the parameter with the greatest influence is Cd(II) initial concentration.

A recent study did not consider reaction time as part of the factors that affect removal of nickel and copper using *Aspergillus* sp. (Pundir et al., 2018). In the current study, it was revealed that the longer the inoculum stays in solution, the more the Cd(II) was removed (Fig. 3). Thus, the influence of reaction time cannot be underestimated; it ranks second, with a percentage contribution of 26.14%.

Also, the influence of inoculum concentration cannot be overlooked as more bacterial colonies are formed with increases in the initial concentration of inoculum. The rate of Cd(II) removal from solution was greatly affected by increasing bacterial isolate populations, leading to an increase in percentage removal for the heavy metal. Thus, inoculum concentration was revealed to be the third most influential parameter, with a percentage contribution of 20.05%. Similar studies have revealed similar results (Kumar et al., 2010; Hamza et al., 2010).

Nevertheless, temperature is another factor that also influences the removal of heavy metal from solution using microorganisms (Ding et al., 2012). In this study, the percentage contribution of temperature to the biosorption process was revealed to be 8.64% and it occupied 4th position. Microbial growth is hindered when subjected to higher temperatures as the protein nature of bacteria is altered under these conditions (Sankarammal et al., 2014). Thus, the higher the temperature, the lower the bacterial efficiency and the more adversely removal of Cd(II) from solution is affected.

Influence of solution pH on heavy metal removal from solution using microorganisms has been documented previously (Dursun et al., 2003; Aksu et al., 2000). The present study revealed pH as having the lowest contribution (5th position), with a percentage influence of 6.07% on Cd(II) biosorption from aqueous solution.

Table 6. Factorial sum of squares and percentage contribution of each factor

Factor	SS_F	ρ_F
A	33.59	6.07
B	47.34	8.64
C	141.14	26.14
D	108.48	20.05
E	182.54	33.87

Solution pH determines the nature of the charge (either positive or negative) on the surface of the bacteria, and thereby determines the nature of the force (repulsion or attraction) between the bacteria and the heavy metal ions.

ANOM technique for process parameter optimization

The significance of S/N ratio plotted in Figs 1–5 was as a tool for process parameter optimization. The optimum condition is measured at factor-level combination where the S/N ratio has the maximum value. Maximum values of S/N ratio were recorded to be 38.93 (Fig. 1), 39.17 (Fig. 2), 39.09 (Fig. 3), 39.20 (Fig. 4) and 38.97 (Fig. 5), where pH, temperature, time, inoculum concentration and Cd(II) initial concentration were 4, 303 K, 4 h, 15 v/v % and 50 mg/L, respectively. Thus, the factor-level combination that gives optimum Cd(II) removal from solution using bacterial isolates from sawdust was A2, B1, C4, D3 and E1. A similar study where only 4 factors were considered presented similar results for optimum biosorption of copper and nickel from solution using *Aspergillus* sp. fungi (Pundir et al., 2018).

In order to confirm the efficacy of bacteria isolates from sawdust in removing Cd(II) from aqueous solution, a laboratory test was conducted at the predicted optimum condition (pH = 4, temperature = 303 K, time = 4 h, inoculum concentration = 15 v/v % and Cd(II) initial concentration = 50 mg/L) to calculate the percentage of heavy metal removal. At these parameter levels, the percentage of Cd(II) removed was 99.53%. This reveals the efficiency of the bacteria used to remove Cd from solution at optimum operating conditions. Table 7 compares the conditions at which optimum removal of Cd(II) was achieved in this study with results from the literature.

Effect of parameter interaction on Cd(II) removal

Figure 6 represents the 3D surface plot showing effects of interaction of pH and temperature (Fig. 6a), pH and time (Fig. 6b), pH and inoculum concentration (Fig. 6c), pH and Cd(II) initial concentration (Fig. 6d), temperature and time (Fig. 6e), temperature and inoculum concentration (Fig. 6f), temperature and Cd(II) initial concentration (Fig. 6g), time and inoculum concentration (Fig. 6h), time and Cd(II) initial concentration (Fig. 6i), inoculum concentration and Cd(II) initial concentration (Fig. 6j), on Cd(II) biosorption from aqueous solution using bacterial isolates from sawdust. A high level of complementary interactive effects was exhibited among the investigated factors at different levels for the biosorption process. The plots were executed via plotting any two independent variables against each other while other variables were held constant. All actual factors were kept constant as Level 1, such that pH, temperature, time, inoculum

concentration and Cd(II) initial concentration values were 2, 303 K, 1 hr, 5 v/v (%) and 50 mg/L, respectively. The percentage of Cd(II) removed from aqueous solution was greater than 76% in each of the plots. This affirms the high efficacy of bacterial isolates from sawdust as an effective biosorbent for Cd(II) removal from aqueous solution.

Bacterial isolate characterization

Fourier transform infrared spectroscopy (FTIR)

Figures 7a and 7b present the FTIR spectra of bacterial isolates from waste sawdust, before and after Cd(II) biosorption from aqueous solution. Presence of sharp peaks is a strong indication that active functional groups are present on the surface of bacterial isolates (Haq et al., 2015). A shift in sharp peaks is a strong indication that biosorption of the contaminant has taken place (Popoola, 2019b). Major sharp peaks observed at 3 583.41; 2 925.74 and 2 396.11 cm^{-1} before Cd(II) removal (Fig. 7a) shifted to 3 436.33; 2 929.5 and 2 355.43 cm^{-1} , respectively, after Cd(II) removal from aqueous solution (Fig. 7b). The major assignment at these respective wavelengths could be attributed to –OH stretching (Chojnacka et al., 2005), suggesting that this functional group is responsible for binding the metallic ions present in solution. Presence of the –OH group on the bacterial surface makes its surface negatively charged, which enhances the removal of positively charged Cd(II) from aqueous solution at a higher solution pH. This is a strong indication of bacteria isolates ability to take up Cd(II) from aqueous solution.

Scanning electron microscopy (SEM)

The SEM image of bacterial isolates from sawdust before (Fig. 8a) and after Cd(II) removal (Fig. 8b) are shown in Fig. 8. A porous morphological nature was shown before Cd(II) removal, which enhances the biosorption of the contaminant from aqueous solution. After the removal, the multiple layers of walls on the bacterial inner surface were seen to be covered with the Cd(II) ion, suggesting the effectiveness of the bacterial isolates as a biosorbent for Cd(II) in aqueous solution (Abou-Taleb et al. 2017).

Bacterial isolate characterization

Table 8 presents the characterization of the bacterial isolates. The isolates were found to be motile when examined under the microscope. The isolates did not ferment glucose, sucrose, mannose and lactose and also exhibited gram-negative reaction attributes. They produce blue-green pigment and responded negatively to methyl red. However, the isolates responded positively to catalase, oxidase and citrate tests (Lennox et al., 2019; Hossain et al., 2013; Zhang et al., 2018).

Table 7. Comparison between Cd removal results in literature with the results of this study at optimum conditions

Adsorbent	pH	Temp.	Time	Inoculum conc.	Contaminant initial conc.	Efficiency	Reference
		(K)	(hr)	v/v (%)	(mg/L)	(%)	
<i>Pseudomonas putida</i>	6	303	1	-	-	71.00	Pardo et al., 2003
<i>Bacillus circulans</i>	7	293	2	0.5	-	63.58	Yilmaz et al., 2005
Fireworks-exposed soil	6	308	12	8	20	90.00	Kumar et al., 2012
<i>Dracaena draca</i>	7	-	-	0.5	10	79.60	Mahmoud et al., 2016
<i>Escherichia coli</i>	8	313	1	0.01	-	68.58	Tafakori et al., 2017
<i>Ulva fasciata</i>	5	298	1	4	200	99.96	El-Naggar et al., 2018b
Bacteria isolates	4	303	4	15	50	99.53	This study

Table 8. Motility, sugar fermentation and biochemical tests of bacterial isolates from sawdust

Test	Motility	Methyl red	Catalase	Glucose	Sucrose	Mannose	Lactose	Pigment	Oxidase	Gram reaction	Citrate
Result	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Blue-green	Positive	Negative	Positive

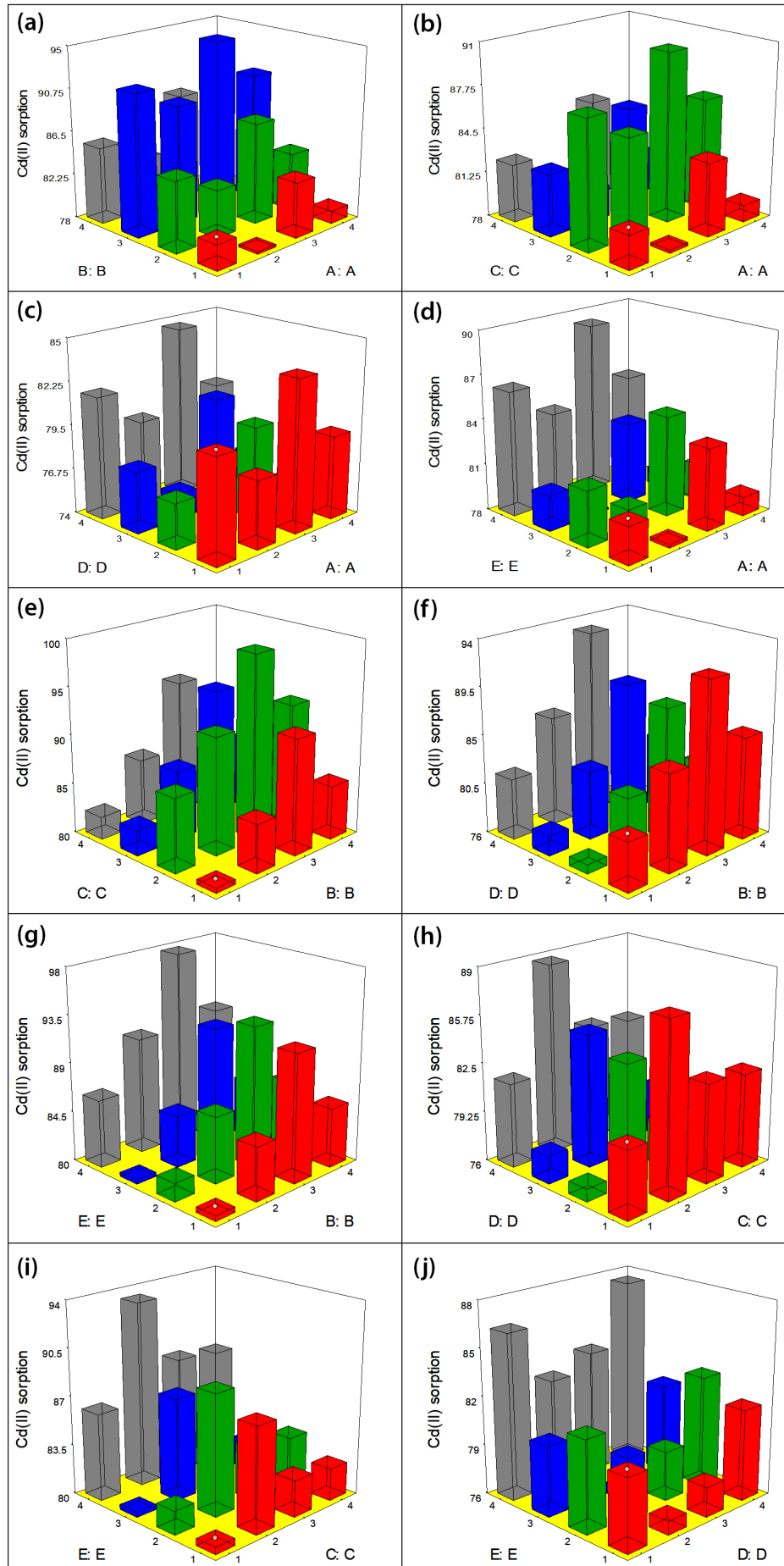


Figure 6. 3D surface plot of parameter interaction for (a) pH and temperature, (b) pH and time, (c) pH and inoculum concentration, (d) pH and Cd(II) initial concentration, (e) temperature and time, (f) temperature and inoculum concentration, (g) temperature and Cd(II) initial concentration, (h) time and inoculum concentration, (i) time and Cd(II) initial concentration, (j) inoculum concentration and Cd(II) initial concentration, on Cd(II) biosorption from aqueous solution using bacterial isolates from sawdust

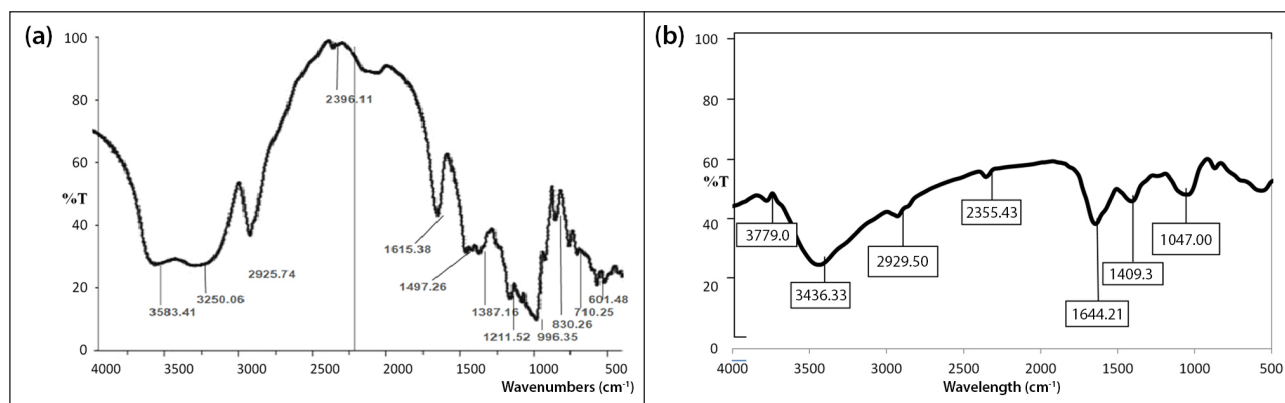


Figure 7. FTIR spectra of bacterial isolates from sawdust: (a) before, and (b) after Cd(II) removal

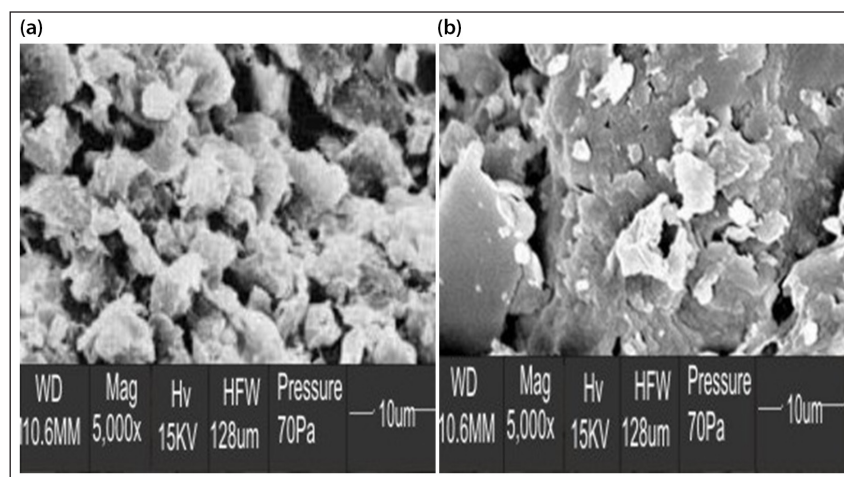


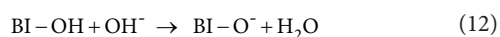
Figure 8. SEM image of bacterial isolates from sawdust: (a) before and (b) after Cd(II) biosorption

Biosorption mechanism

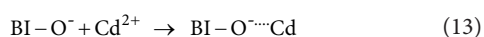
Different mechanisms of heavy metal biosorption from aqueous solution have been proposed and developed (Wu et al., 2010). In this study, the proposed mechanism of Cd(II) removal from solution using bacterial isolates from sawdust includes electrostatic interaction, surface complexation and ion exchange.

Electrostatic interaction

The FTIR analysis has revealed the presence of –OH groups on the bacterial isolates' (BI) surface, with three sharp peaks revealed indicating –OH stretching. The cadmium salt is a bivalent positively charged heavy metal ion with a strong affinity for negatively charged surface biosorbent. At high pH, the surface of the bacteria is more negatively charged and, thus, loss of protons (deprotonation) occurs, with formation of water, as presented in Eq. 12.



Strong electrostatic forces then prevailed which enhanced the strong attraction between Cd(II) and bacterial isolates, as presented in Eq. 13.



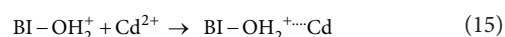
Surface complexation

At low pH, the bacterial surface becomes more positively charged, and thus a gain of protons occurs (protonation), as presented in Eq. 14. As such, the protonated bacteria forms complexes at the cell surface.



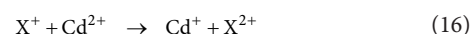
The Cd(II) salt becomes more active as more bivalent ions are

formed. More complexes are formed on the bacterial surface as shown in Eq. 15.



Ion exchange

Under this mechanism, the counter ions (X^+) present in polysaccharides (a main constituent of bacterial isolates' cell wall) are exchanged with bivalent cadmium ions resulting from covalent bonding between the two, as presented in Eq. 16.



Other mechanisms

Other proposed mechanisms, as suggested by previous studies, could be interaction with oxygen-containing functional groups (Fawzy et al., 2019); precipitation interaction involving inorganic minerals such as carbonates, phosphates, and silicates (Huang et al., 2018); and bioaccumulation and pore surface physical agglomeration (Iqbal et al., 2009).

CONCLUSIONS

The results obtained from this present study revealed bacterial isolates from sawdust to be an effective biosorbent for the biosorption of Cd(II) from aqueous solution. Taguchi experimental design with L_{16} 4-level factors orthogonal array was utilized to investigate the optimal process parameters that would give maximum percentage removal of Cd(II) from aqueous solution. The confirmatory experiment conducted at the predicted optimal conditions (pH = 4, temperature = 303 K, time = 4 h, inoculum concentration = 15 v/v % and Cd(II) initial concentration = 50 mg/L) revealed 99.53% removal for Cd(II).

Analysis of variance revealed the order of factors' influence to be Cd(II) initial concentration (33.87%) > time (26.14%) > inoculum concentration (20.05%) > temperature (8.64%) > pH (6.07%). Effect of parameter interaction revealed the percentage of Cd(II) removed from aqueous solution to be greater than 76% in each of the 3D surface plots. Fourier transform infrared analysis revealed the presence of -OH on the bacterial surface as the main active functional group enhancing Cd(II) biosorption. Scanning electron microscopy revealed the presence of small cavities on the bacterial surface, with a porous inner multilayer. Mechanisms of biosorption were proposed to be electrostatic interaction, surface complexation and ion exchange. With reference to these observations, bacterial isolates from sawdust could effectively be applied as biosorbent for Cd(II) removal from aqueous solution.

REFERENCES

- ABDULLA KJ, ALI SA, GATEA IH, HAMEED NA and MAIED SK (2019) Bio-degradation of crude oil using local bacterial isolates. *IOP Conf. Series: Earth and Environmental Science*. **388**. 012081. <https://doi.org/10.1088/1755-1315/388/1/012081>
- ABOU-TALEB KAA, EWEDA WE and MIRA HI (2017) Optimization of chromium and copper ions uptake by *Aspergillus terreus* strain using different techniques. *Adv. Food Sci. Eng.* **1** (1) 39–57. <https://doi.org/10.22606/afse.2017.11005>
- AKA RJN and BABALOLA OO (2016) Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. *Int. J. Phytoremed.* **8** 200–209. <https://doi.org/10.1080/15226514.2015.1073671>
- AKSU Z and DÖNMEZ G (2000) The use of molasses in copper(II) containing wastewaters: effects on growth and copper(II) bioaccumulation properties of *Kluyveromyces marxianus*. *Process Biochem.* **36** 451–458. [https://doi.org/10.1016/S0032-9592\(00\)00234-X](https://doi.org/10.1016/S0032-9592(00)00234-X)
- AMINI M, YOUNESI H, BAHRAMIFAR N, LORESTANI AAZ, GHORBANI F, DANESHI ALI and SHARIFZADEH M (2008) Application of response surface methodology for optimization of lead biosorption in an aqueous solution by *Aspergillus niger*. *J. Hazardous Mater.* **154** 694–702. <https://doi.org/10.1016/j.jhazmat.2007.10.114>
- ATLAS RM and BARTHA R (1973) Stimulated biodegradation of oil slicks using oleophilic fertilizers. *Environ. Sci. Technol.* **7** 538–541. <https://doi.org/10.1021/es60078a005>
- BARRADO M, VEGA R, PARDON P and GRANDE JLD (1996) Optimization of a purification method for metal-containing wastewater by use of a Taguchi experimental design. *Water Res.* **30** 2309–2314. [https://doi.org/10.1016/0043-1354\(96\)00119-4](https://doi.org/10.1016/0043-1354(96)00119-4)
- BOUAZIZI H, JOUILI H, GEITMANN A and FERJANI EEI (2010) Copper toxicity in expanding leaves of *Phaseolus vulgaris* L.: antioxidant enzyme response and nutrient element uptake. *Ecotox. Environ. Saf.* **73** 1304–1308. <https://doi.org/10.1016/j.ecoenv.2010.05.014>
- CHEESBROUGH M (1985) Medical laboratory manual for tropical countries. *Microbiology*. **2** 248–264.
- CHELLAIAH ER (2018) Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: a minireview. *Appl. Water Sci.* **8** 154. <https://doi.org/10.1007/s13201-018-0796-5>
- CHOJNACKA K, CHOJNACKI A and GORECKA H (2005) Biosorption of Cr(III), Cd(II) and Cu(II) ions by blue-green algae *Spirulina* sp. *Chemosphere*. **59** 75–84. <https://doi.org/10.1016/j.chemosphere.2004.10.005>
- DANESHVAR N, KHATAEE AR, RASOULIFARD MH and POURHASSAN M (2007) Biodegradation of dye solution containing Malachite Green, optimization of effective parameters using Taguchi method. *J. Hazardous Mater.* **143** 214–219. <https://doi.org/10.1016/j.jhazmat.2006.09.016>
- DING Y, JING D, GONG H, ZHOU L and YANG X (2012) Biosorption of aquatic cadmium(II) by unmodified rice straw. *Bioresour. Technol.* **114** 20–25. <https://doi.org/10.1016/j.biortech.2012.01.110>
- DURSUN AY, USLU G, CUCI Y and AKSU Z (2003) Bioaccumulation of copper (II), lead (II) and chromium (VI) by growing *Aspergillus niger*. *Process Biochem.* **38** 1647–1651. [https://doi.org/10.1016/S0032-9592\(02\)00075-4](https://doi.org/10.1016/S0032-9592(02)00075-4)
- EL-NAGGAR NE, HAMOUDA RA, MOUSA IE, ABDEL-HAMID MS and RABEI NH (2018a) Biosorption optimization, characterization, immobilization and application of *Gelidium amansii* biomass for complete Pb²⁺ removal from aqueous solutions. *Sci. Rep.* **8** 13456. <https://doi.org/10.1038/s41598-018-31660-7>
- EL-NAGGAR NE, HAMOUDA RA, MOUSA IE, ABDEL-HAMID MS and RABEI NH (2018b) Statistical optimization for cadmium removal using *Ulva fasciata* biomass: Characterization, immobilization and application for almost-complete cadmium removal from aqueous solutions. *Sci. Rep.* **8** 12456. <https://doi.org/10.1038/s41598-018-30855-2>
- ENGIN AB, OZDEMIR O, TURAN M and TURAN AZ (2008) Color removal from textile dye bath effluents in a zeolite fixed bed reactor: determination of optimum process conditions using Taguchi method. *J. Hazardous Mater.* **159** 348–353. <https://doi.org/10.1016/j.jhazmat.2008.02.065>
- FAWZY M, NASR M, ABDEL-RAHMAN AM, HOSNY G and ODHABA BR (2019) Techno-economic and environmental approaches of Cd²⁺ adsorption by olive leaves (*Olea europaea* L.) waste. *Int. J. Phytoremed.* **21** (12) 1205–1214. <https://doi.org/10.1080/15226514.2019.1612848>
- GHANI JA, JAMALUDIN H, RAHMAN MNA and DEROS BM (2013) Philosophy of Taguchi approach and method in design of experiment. *Asian J. Sci. Res.* **6** (1) 27–37. <https://doi.org/10.3923/ajsr.2013.2737>
- GUO J, ZHENG XD, CHEN QB, ZHANG L and XU XP (2012) Biosorption of Cd(II) from aqueous solution by *Pseudomonas plecoglossicida*. *Curr. Microbiol.* **65** 350–355. <https://doi.org/10.1007/s00284-012-0164-x>
- HAMZA SM, AHMED HF and MOHAMMAD EAM (2010) Optimization of cadmium, zinc and copper biosorption in an aqueous solution by *Saccharomyces cerevisiae*. *J. Am. Sci.* **6** 597–604.
- HAQ F, BUTT M, ALI H and CHAUDHARY HJ (2015) Biosorption of cadmium and chromium from water by endophytic *Kocuria rhizophila*: Equilibrium and kinetic studies. *Desalin. Water Treat.* **20** (15) 1–13.
- HARITASH AK and KAUSHIK CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *J. Hazardous Mater.* **169** 1–15. <https://doi.org/10.1016/j.jhazmat.2009.03.137>
- HE HJ, XIANG ZH and CHEN XJ (2018) Biosorption of Cd (II) from synthetic wastewater using dry biofilms from biotrickling filters. *Int. J. Environ. Sci. Technol.* **15** (7) 1491–1500. <https://doi.org/10.1007/s13762-017-1507-8>
- HOSSAIN MG, SAHA S, RAHMAN MM, SINGHA JK and MAMUN AA (2013) Isolation, identification and antibiogram study of *Pseudomonas aeruginosa* from cattle in Bangladesh. *J. Vet. Adv.* **3** (7) 180–185. <https://doi.org/10.5455/jva.20130717123841>
- HRISTOV J (2014) The role and use of water in agriculture in the Western Balkans: The case of Macedonia. PhD thesis, Swedish University of Agricultural Sciences.
- HU JL, HE XW, WANG CR, LI JW and ZHANG CH (2012) Cadmium adsorption characteristic of alkali modified sewage sludge. *Bioresour. Technol.* **121** 25–30. <https://doi.org/10.1016/j.biortech.2012.06.100>
- HUANG J, LIU Z, LI S, XU B, GONG Y, YANG Y and SUN H (2016) Isolation and engineering of plant growth promoting rhizobacteria *Pseudomonas aeruginosa* for enhanced cadmium bioremediation. *J. Gen. Appl. Microbiol.* **62** 258–265. <https://doi.org/10.2323/jgam.2016.04.007>
- HUANG Q, CHEN Y, YU H, YAN L, ZHANG J, DU BWB and XING L (2018) Magnetic graphene oxide/MgAl-layered double hydroxide nanocomposite: One-pot solvothermal synthesis, adsorption performance and mechanisms for Pb²⁺, Cd²⁺ and Cu²⁺. *Chem. Eng. J.* **341** 1–9. <https://doi.org/10.1016/j.cej.2018.01.156>
- IQBAL M, SAEED A and ZAFAR SI (2009) FTIR spectrophotometry, kinetics and adsorption isotherms modeling, ion exchange, and EDX analysis for understanding the mechanism of Cd²⁺ and Pb²⁺ removal by mango peel waste. *J. Hazardous Mater.* **164** (1) 161–171. <https://doi.org/10.1016/j.jhazmat.2008.07.141>
- JACOB JM, KARTHIK C and SARATALE RG (2018) Biological approaches to tackle heavy metal pollution: A survey of literature. *J. Environ. Manage.* **217** 56–70. <https://doi.org/10.1016/j.jenvman.2018.03.077>

- JAVANBAKHT V, ALAVI SA and ZILOUEI H (2014) Mechanisms of heavy metal removal using microorganisms as biosorbent. *Water Sci. Technol.* **69** (9) 1775–1787. <https://doi.org/10.2166/wst.2013.718>
- KASSAB DM and ROANE TM (2006) Differential responses of a mine tailings *Pseudomonas* isolate to cadmium and lead exposures. *Biodegradation.* **17** 379–387. <https://doi.org/10.1007/s10532-005-9010-1>
- KUMARA, BISHT BS and JOSHI VD (2010) Biosorption of heavy metals by four acclimated microbial species, *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Aspergillus niger*. *J. Biol. Environ. Sci.* **4** 97–108.
- LENNOX JA, ASITOK A, JOHN GE and ETIM BT (2019) Characterization of products from sawdust biodegradation using selected microbial culture isolated from it. *Afr. J. Biotechnol.* **18** (29) 857–864.
- MCAFADDEN JF (2000) *Biochemical Tests for Identification of Medical Bacteria* (3rd edn.). The Williams & Wilkins Co., Baltimore. 689–691.
- MAHMOUD AED, FAWZY M and RADWA AA (2016) Optimization of cadmium (Cd²⁺) removal from aqueous solutions by novel biosorbent. *Int. J. Phytoremed.* **18** (6) 619–625. <https://doi.org/10.1080/15226514.2015.1086305>
- MARQUES APGC, RANGEL AOSS and CASTRO PML (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Crit. Rev. Environ. Sci. Technol.* **39** (8) 622–654. <https://doi.org/10.1080/10643380701798272>
- MKADMI Y, BENABBI O, FEKHAOU M, BENAKKAM R, BIJJOU W, ELAZZOUI M, KADOURRI M and CHETOUANI A (2018) Study of the impact of heavy metals and physico-chemical parameters on the quality of the wells and waters of the Holcim area (Oriental region of Morocco). *J. Mater. Environ. Sci.* **9** (2) 672–679.
- MOUSAV S, YAGHMAEI A, JM VOSSOUGH I and GHOBADI Z (2007) Optimization of ferrous biooxidation rate in a packed bed bioreactor using Taguchi approach. *Chem. Eng. Process.* **46** 935–940. <https://doi.org/10.1016/j.cep.2007.06.010>
- NAGENDRAPPA G, BHASKAR CV and KUMAR K (2010) Assessment of heavy metals in water samples of certain locations situated around Tumkur, Karnataka India. *J. Chem.* **7** (2) 349–352. <https://doi.org/10.1155/2010/415150>
- PARDO R, HERGUEDAS M, BARRADO E and VEGA M (2003) Biosorption of cadmium, copper, lead and zinc by inactive biomass of *Pseudomonas putida*. *Anal. Bioanal. Chem.* **376** (1) 26–32. <https://doi.org/10.1007/s00216-003-1843-z>
- PATRÓN-PRADO M, ACOSTA-VARGAS B, SERVIERE-ZARAGOZA E and MÉNDEZ-RODRÍGUEZ L (2010) Copper and cadmium biosorption by dried seaweed *Sargassum sinicola* in saline wastewater. *Water, Air Soil Pollut.* **210** 197–202. <https://doi.org/10.1007/s11270-009-0241-3>
- PÉREZ-RAMA M, TORRES E, SUÁREZ C, HERRERO C and ABALDE J (2010) Sorption isotherm studies of Cd(II) ions using living cells of the marine microalga *Tetraselmis suecica* (Kyllin) Butch. *J. Environ. Manage.* **91** 2045–2050. <https://doi.org/10.1016/j.jenvman.2010.05.014>
- POPOOLA LT (2019a) Tetracycline and sulfamethoxazole adsorption onto nanomagnetic walnut shell-rice husk: isotherm, kinetic, mechanistic and thermodynamic studies. *Int. J. Environ. Anal. Chem.* **100** (9) 1021–1043. <https://doi.org/10.1080/03067319.2019.1646739>
- POPOOLA LT (2019b) Nano-magnetic walnut shell-rice husk for Cd(II) sorption: design and optimization using artificial intelligence and design expert. *Heliyon.* **5** 1153. <https://doi.org/10.1016/j.heliyon.2019.e02381>
- POPOOLA LT, ADERIBIGBE TA, YUSUFF AS and MUNIR MM (2018) Brilliant green dye adsorption onto composite snail shell–rice husk: Adsorption isotherm, kinetic, mechanistic and thermodynamics analysis. *Environ. Qual. Manage.* **1** 16. <https://doi.org/10.1002/tqem.21597>
- PUNDIR R, CHARY GHVC and DASTIDAR MG (2018) Application of Taguchi method for optimizing the process parameters for the removal of copper and nickel by growing *Aspergillus* sp. *Water Resour. Ind.* **20** 83–92. <https://doi.org/10.1016/j.wri.2016.05.001>
- RAJESHKUMAR R, SAHU S and AGHARWAL JR (2012) Biosorption of cadmium (II) ions by the cadmium tolerant bacteria isolated from the chemical exposed soil of fireworks industry. *J. Pure Appl. Microbiol.* **6** (2) 781–787.
- RAO MM, RAMESH A, RAO GPC and SESHAIHAH K (2006) Removal of copper and cadmium from the aqueous solutions by activated carbon derived from *Ceiba pentandra* hulls. *J. Hazardous Mater.* **129** (1) 123–129. <https://doi.org/10.1016/j.jhazmat.2005.08.018>
- REHMAN R, FAROOQ S and MAHMUD T (2019) Use of agro-waste *Musa acuminata* and *Solanum tuberosum* peels for economical sorptive removal of emerald green dye in ecofriendly way. *J. Clean. Prod.* **206** 819–826. <https://doi.org/10.1016/j.jclepro.2018.09.226>
- SALMAN M, REHMAN R, FAROOQ U, TAHIR A and MITU L (2020) Biosorptive removal of cadmium(ii) and copper(ii) using microwave-assisted thiourea-modified *sorghum bicolor* agrowaste. *J. Chem.* **2020** Article ID 8269643. 11 pp. <https://doi.org/10.1155/2020/8269643>
- SANKARAMMAL M, THATHEYUS A and RAMYA D (2014) Bioremoval of cadmium using *Pseudomonas fluorescens*. *Open J. Water Pollut. Treat.* **1** 92–100. <https://doi.org/10.15764/WPT.2014.02010>
- SHARMA PK, BALKWILL DL, FRENKEL A and VAIRAVAMURTHY MA (2000) A new *Klebsiella planticola* strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. *Appl. Environ. Microbiol.* **66** 3083–3087. <https://doi.org/10.1128/AEM.66.7.3083-3087.2000>
- SINGH B, ALLOWAY BJ and BOCHEREAU FJM (2000) Cadmium sorption behaviour of natural and synthetic zeolites. *Commun. Soil Sci. Plant Anal.* **3117** 2775–2786. <https://doi.org/10.1080/00103620009370626>
- SRIDEVI V, LAKSHMI MVVC, SWAMY AVN and RAO MN (2011) Implementation of response surface methodology for phenol degradation using *Pseudomonas putida* (NCIM 2102). *J. Bioremed. Biodegrad.* **2** (2) 1–7. <https://doi.org/10.4172/2155-6199.1000121>
- TAFAKORI V, ZADMARD R, TABANDEH F, AMOOZEGAR MA and AHMADIAN G (2017) Equilibrium isotherm, kinetic modeling, optimization, and characterization studies of cadmium adsorption by surface-engineered *Escherichia coli*. *Iran. Biomed. J.* **21** (6) 380–391.
- WU G, KANG H, ZHANG X, SHAO H, CHU L and RUAN C (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: Issues, progress, eco-environmental concerns and opportunities. *J. Hazardous Mater.* **174** (1–3) 1–8. <https://doi.org/10.1016/j.jhazmat.2009.09.113>
- WU M, LIU H and YANG C (2019) Effects of pretreatment methods of wheat straw on adsorption of Cd (II) from waterlogged paddy soil. *Int. J. Environ. Res. Public Health.* **16** (2) 205–211. <https://doi.org/10.1016/j.jhazmat.2009.09.113>
- YALÇIN S, SEZER S and APAK R (2012) Characterization and lead (II), cadmium(II), nickel(II) biosorption of dried marine brown macroalgae *Cystoseira barbata*. *Environ. Sci. Pollut. Res.* **19** 3118–3125. <https://doi.org/10.1007/s11356-012-0807-2>
- YAN G and VIRARAGHAVAN T (2003) Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Res.* **37** 4486–4496. [https://doi.org/10.1016/S0043-1354\(03\)00409-3](https://doi.org/10.1016/S0043-1354(03)00409-3)
- YILMAZ EI and ENSARI NY (2005) Cadmium biosorption by *Bacillus circulans* strain EBI. *World J. Microbiol. Biotechnol.* **21** (5) 777–779. <https://doi.org/10.1007/s11274-004-7258-y>
- ZHANG H, KRAFFT T, GAO D, ZHENG G and CAI L (2018) Lignocellulose biodegradation in the biodrying process of sewage sludge and sawdust. *Drying Technol.* **36** (3) 316–324. <https://doi.org/10.1080/07373937.2017.1326502>
- ZOUBOULIS A, LOUKIDOU M and MATIS K (2004) Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochem.* **39** 909–916. [https://doi.org/10.1016/S0032-9592\(03\)00200-0](https://doi.org/10.1016/S0032-9592(03)00200-0)