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1 **Optimizing the biosorption of Bi³⁺ ions by *Streptomyces rimosus* using experimental**
2 **design and applicability in kinetics and isotherms modeling**

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10

11 **Abstract**

12 This investigation seeks to analyze bismuth biosorption onto *Streptomyces rimosus* in solutions
13 following optimization by using Box-Behnken Design (BBD). Based on efficient method, three
14 significant parameters including pH, temperature, and initial bismuth (Bi³⁺) concentration in a batch
15 system were studied by using Design of Experiment (DOE). Central composite second order response
16 surface methodology (RSM) accomplishes construction of model biosorption (R %) and operating
17 condition. Using this statistical–mathematical method leads to obtain a second-order equation for the
18 bismuth removal. The regression equation was obtained by using Design Expert 7.0 software. The
19 numerical optimization shows great biosorption percentage (>97%) at pH 8.0, 30°C, and 30 mg L⁻¹ for
20 bismuth. The quadratic models exhibited higher R² values, significant p-values, and insignificant lack-
21 of-fit *p-values* that all confirm high adequacy for predicting the response. Both mathematical empirical
22 models due to their high correlation coefficients (R²) of 0.9997 are suitable for predicting biosorption
23 trend bismuth solution. The closeness of predicted values and experimental value also support this
24 conclusion. The pseudo-second order kinetic model adequately described the kinetic data. The
25 Langmuir isotherm model described the process of Bi³⁺ uptake better than the other models. The
26 maximum biosorption capacity of the biosorbent was found to be 38.91 mg g⁻¹ for bismuth
27 biosorption. The possible interactions between biosorbents and bismuth were also evaluated by
28 Fourier transform infrared (FT-IR) spectroscopy analysis.

29
30 **Keywords:** Biosorption; Bi³⁺; Design of experiments; Equilibrium isotherm; Kinetic; *Streptomyces*
31 *rimosus*.
32

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33 **1. Introduction**

34 Environmental problems associated with heavy metals are very difficult to be solved in contrast to
35 organic Matters because incineration or biodegradation can transform the latter.¹ As a fact, most of
36 heavy metals have toxic Effects on living organisms when transcending a certain concentration.
37 Besides, some heavy metals are being Subject to bioaccumulation and may pose a risk to human
38 health when transferred to the food chain.² Bismuth and its compounds have been used in many
39 variance areas such as cosmetic products, lubricatingoils, medicines, pigments, electronics,
40 semiconductors, alloys industry and in recycling of uranium nuclear fuels.^{3, 4} Neuropathology,
41 osteoarthropathy, nephropathy and hepatitis have been ascribed to bismuth compounds as toxic effects
42 on humans.⁵ Therefore, bismuth species are included in the list of potential toxins.⁶⁻⁸ The
43 Conventional processes such as ion-exchange; electrolysis and biosorption for heavy metal removal
44 often are neither effective nor economical.^{9,10} Hence there is a great need for an alternative technique,
45 which is both economical and efficient. Biosorption is an innovative technology and has distinct
46 preferences over conventional methods being currently employed.¹¹ As an alternative to traditional
47 methods, its promising results are now being considered for application by the scientific community.
48 Biosorption is either metabolism independent, such as physical or chemical biosorption onto the cell
49 wall, or metabolism depended, such as transport, internal compartmentalization, and extra cellular
50 precipitation by metabolites. Biosorption has other advantages over conventional treatment methods as
51 low cost, a minimal amount of chemical and biological sludge and the potentiality of biosorbent
52 regeneration and metal recovery.⁹ Microorganisms can adjust and grow under divers extreme
53 conditions of pH, temperature and nutrient availability, as well as high metal concentrations.¹²⁻¹⁵
54 Generally, microbial biomasses have evolved various measures to respond to heavy metal stress via
55 processes such as transport across the cell membrane, biosorption to cell walls, entrapment in
56 extracellular capsules, as well as precipitation and transformation of metals.¹⁶⁻¹⁸ Recent investigations
57 by various biosorbents groups have shown the impressive biosorption capacities for a range of heavy
58 metal ions. But no information are available for bismuth (Bi^{3+}) biosorption from aqueous solutions by
59 microorganism until now.

60 The design and efficient operation of biosorption processes require equilibrium data for using in
61 kinetic and mass transfer models. These models can then be used to predict the performance of the
62 biosorption contact processes under a range of operating conditions.¹⁹

63 The purpose of this study was to investigate the biosorption characteristics of bismuth (Bi^{3+}) ions on
64 *Streptomyces rimosus*. The effects of operating parameters such as pH, temperature, and initial metal
65 ion on the biosorption of bismuth (Bi^{3+}) ions were analyzed using RSM.

66

67 **2. Experimental**

68 **2.1. Biosorbent preparation**

69 At first, *Streptomyces rimosus* obtained from the Iranian Research Organization for Science and
70 Technology (IROST) was cultured on Trypaic Soy Broth (TSB) and incubated for 24 h in 30 °C
71 .then,1 colony was dissolved in a 100 mL Erlenmeyer flask containing 25 mL of TSB and placed on
72 rotator shaker for 24 h in 30 °C in order to grow bacteria.²⁰⁻²²

73

74 **2.2. Materials and apparatus**

75 Determination of the metal ions in sample was carried out by the GBC model Avanta flame atomic
76 absorption spectrometer (Sidney, Australia) fitted with bismuth hollow cathode lamps and air
77 acetylene flame. The infrared spectra were obtained using a Bomem FT-IR spectrophotometer
78 (Canada) to identify the functional groups. pH adjustments were carried out by the Metrohm pH-meter
79 model 827 (Herisau, Switzerland). Response surface analysis was performed by the Design-Expert®
80 Software (Version 7.0, Stat-Ease Inc., USA). The significances of all terms in the polynomial equation
81 were analyzed statistically by computing the F-value at a probability (p) of 0.05. The Stock Bi³⁺
82 solution of 1000 mg L⁻¹ which was prepared by bismuth nitrate penta-hydrate (Merck, Germany).
83 Then, the solution was sterilized by autoclaving at a pressure of 1.5 atoms and a temperature of 121°C
84 for 30 min

85

86 **2.3. Batch biosorption studies**

87 In the batch biosorption experiments 1mL of bacteria solution was added to 25 mL of each bismuth
88 solution with shaking rate of 150 rpm, equilibrium was reached after 48h, the required pH value of the
89 solutions was adjusted with 0.1 mol L⁻¹ HCl or NaOH solutions.²⁰⁻²³ Samples were taken at given time
90 intervals and were centrifuged at 6000 rpm, in order to isolate and measure dry weight absorbent.
91 Then, the filtered supernatant was used to determine bismuth concentration with atomic absorption
92 spectrophotometer.

93 The amount of Biosorption onto bacterial biomass were determined from the difference between the
94 Bi added to the solution and the Bi³⁺ remaining in the solution after 48 h. Blank samples were also
95 examined to ensure no biosorption had taken place on the walls of the apparatus used. Each
96 experiment was repeated three times to confirm the results. The Bi³⁺ uptake was calculated by using
97 the following Eq:²⁴

98

$$99 \quad q_e = \frac{(C_i - C_e)V}{m} \quad (1)$$

100

101 Where q_e denotes the specific metal biosorption (mg g⁻¹), C_i and C_e are the initial and final Bi³⁺
102 concentration (mg L⁻¹) in the solution, respectively. V is the volume of aqueous solution (L) and m is
103 dry weight of biomass for biosorption (g).

104

105 **2.4. Design of experiment study of operational parameters**

106 RSM is a useful technique for development and optimization of biosorption process.²⁵ The main
107 advantages of RSM are the reduced numbers of experimental trials needed to evaluate multiple
108 parameters and their interactions and it is useful for developing, improving, and optimizing process.^{26,}
109 ²⁷ By careful design of experiments, the objective is to optimize a response (output variable) which is
110 influenced by several independent variables (input variables).²⁸ An experiment is a series of tests, or
111 the runs, in which changes are made in the input variables in order to identify the reasons for changes
112 in the output response.^{29,30} One of the most popular design of experiment applied in RSM technique is
113 Box Behnken design (BBD).³¹

114 In the present study, three parameters (initial bismuth concentration, pH and Temperature) were
115 studied using BBD model with three levels in the experimental design model, metal ion concentration
116 (20-40 mg L⁻¹), pH (7.0-9.0) and temperature (25-35°C), were taken as input variables. Bi³⁺ by the
117 biosorbent was taken as the response of the system. The experimental design matrix derived from the
118 BBD model is shown in Table 1. Bismuth adsorbed (mg L⁻¹) by the biosorbent indifferent
119 experimental conditions based on the experimental design matrix was estimated, the results of which
120 have also been included in the same table.

121 The data were subjected to analysis of variance and the coefficient of regression (R²) was calculated to
122 find out the advantageous fit of the model. The quality of the fit of quadratic model was determined
123 from correlation coefficient (R²) value adjusted coefficient of determination (R_{adj}²) which is a criterion
124 to express quality of fitting. The e F-value (Fisher variation ratio), probability value (Prob> F),
125 standard deviation (SD), coefficient of variation (CV %) and adequate precision (AP) are criteria for
126 judgment about quality and applicability of model.³²

127

128 **3. Results and discussion**

129 **3.1. Effect of contact time**

130 As the biosorption process proceeds, the sorbed solute tends to desorb back into the solution.
131 Eventually, the rates of biosorption and desorption will attain an equilibrium state. When the system
132 reaches the sorption equilibrium, no further net biosorption occurs. The effect of contact time at
133 greatest contribution on removal percentage at 30 mg L⁻¹ of bismuth on their biosorption onto the
134 bacteria is presented in Fig. 1. When Bismuth was adsorbed in the first 16–18 h, further time did
135 not increase biosorption rate and reached equilibrium around 48 h.

136

137 **3.2. Statistical analysis**

138 The results of BBD experiments for studying the effects of three independent variables along with the
139 predicted mean and observed responses were shown in Table 1. The application of RSM gave the level
140 of Bi³⁺ biosorption as the function of the initial solution pH (A), Temperature (B) and initial Bi³⁺

141 concentration (C). Based on multiple regression analysis, second-order quadratic polynomial equation
142 for predicted response Y ($R\% \text{Bi}^{3+}$), in terms of coded as well actual values which is expressed as:

143

$$144 \quad y = 97.6 - 10.4A - 8.3B - 7.1C + 7.3AB + 3.5AC + 3.8BC - 36.6A^2 - 44.1B^2 - 29.4C^2 \quad (2)$$

145

146 Where y is the removal rate of Bi^{3+} , A , B and C are the independent variables. F -statistic and P -value
147 were used to determine the significance of each coefficient. The F -statistic of the model was 26200
148 corresponding to P -value (less than 0.0001), which indicated that the model was adequate. The “*lack*
149 *of fit F-value*” of 0.2049 proves low contribution of lack of fit compare to pure error that non-directly
150 suggest suitability and efficiency of model for well representation of experimental data (Table 2). The
151 model R^2 of 0.9997 suggests sample variation of 99.97% for biosorption is ascribed to the independent
152 variables and only 0.03% of the total variation is not described by the model. The adjusted R^2 (0.9999)
153 was sufficiently high to indicate the significance of the model. It has been suggested that R^2 should be
154 at least 80% for a good fit of a mode.³³ Quadratic model was selected for model development as
155 suggested by the software (Table 3). The lower coefficient of variation (CV%) value is an adequate
156 indication of precision and reliability of the experiments.^{26, 34} A low CV% of 1-1.5% suggests a good
157 precision and higher reliability of the models for predicting experimental results. Adequate precision
158 indicates the signal to noise ratio greater than 4 and proves its suitability.³⁵ A ratio 440.0 for Bi^{3+}
159 confirms adequate signal.

160 Fig. 2a presents the plot of the actual values of biosorption of Bi^{3+} vs. predicted values using the model.
161 Based on Fig. 2a, the suitable fitness between experimental data and predicted values could be
162 revealed. The plot of studentized residual versus run number (Fig. 2b) shows that residuals were
163 scattered randomly around ± 2.00 , which indicated a minimal deviation at the fitted value from the
164 observed value.

165

166 **3.3. Interactive effects of two variables**

167 The combined effect of pH and temperature (Fig. 3a) reveal that the biosorption efficiency improved
168 following raising pH from 6.0 to 7.0 that is probably due to the negative surface charge of black cumin
169 at high pH values that following electrostatic interactions via biosorption through different mechanism
170 adsorbed bismuth. The low biosorption in acidic solutions is related to competition between hydrogen
171 and dyes for biosorbent active sites. At high temperatures, the sorbed bismuth amount decreased with
172 raising temperature. The increase in uptake with decreasing temperature is due to large value of
173 diffusion coefficient and sorbate transport enhancement within the pores of the sorbent. The decrease
174 in biosorption at lower temperature (cooler media) is related to weakening of adsorptive forces
175 between the active sites of the adsorbents and adsorbate species and also between the adjacent
176 molecules of the adsorbed phase. On the other hand, according to Arrhenius equation at lower

177 temperature a decrease in mass transfer and diffusion coefficient was observed. As can be seen from
178 Fig. 3a the maximum dyes biosorption was obtained at pH 8.0 and temperature of 30 °C.

179

180 **3.4. Optimization of operational parameters using Desirability Functions (DF)**

181 Numerical optimization is based on selection of desired goal for each factor and response. A minimum
182 and a maximum level had to be provided for each parameter admitted in this study, while curvature of
183 the response surfaces curve and their combination with desirability function sometimes lead to
184 appearance of some maximum. Starting from various points in the design, improved the chances of
185 detecting the “best” local maximum.^{36,37} A multiple response method was applied for optimization of
186 any combination of three factors like pH (7.0-9.0) and initial bismuth concentration (20-40 mg L⁻¹)
187 based on achievement of conditions for reaching the best ideal response (100%). Fig.4 shows a ramp
188 desirability that was generated from 10 optimum points via numerical optimization. The best local
189 maximum was found to be at pH of 8.0, temperature of 30 °C, and initial Bi³⁺ concentration of 30
190 mg L⁻¹ a desirability value of 1.00 indicates that the estimated function can well represent the
191 experimental model and desired conditions.

192

193 **3.5. Fourier transform infrared (FT-IR) spectroscopic study**

194 In order to identify which functional groups might be involved in bismuth biosorption, a FT-IR study
195 was carried out. Fig.5 shows The IR spectrum of metal-free and metal-loaded biomass, of bottom to
196 top respectively and displaying a number of absorption peaks and indicating the complex nature of
197 the biomass examined. Table 5 shows assignment of the main characteristic absorption bands for
198 each of four structures. The FTIR analysis results showed the peak intensity changed in response to
199 dosing with heavy metals. Table 3 and Fig. 5 showed the changes in the intensity of bands at different
200 regions that are related to functional groups of –OH, NH, –CN, –CH and –CO on the bacterial cell
201 walls. Thus, it is concluded that these groups play an important role in uptake of bismuth.

202

203 **3.6. Energy dispersive analysis of X-rays (EDAX)**

204 Fig. 6a depicts EDAX spectra of *Streptomyces rimosus* and Bi-*Streptomyces rimosus* . The bismuth
205 peak present in the EDAX spectrum of the bismuth sorbed *Streptomyces rimosus* composite depicted
206 in Fig. 6b confirms the biosorption of bismuth. The quantitative elemental composition of biosorbent
207 after metal ion sorption is listed in the respective figure.

208

209 **3.7. Effect of initial bismuth concentration**

210 The initial metal concentration provides an important driving force to overcome the diffusive mass
211 transfer resistance of all molecules between the sorbate and sorbent.⁹ Therefore, in the present study,
212 the initial concentration of bismuth was varied from 10 to 100 mg L⁻¹ at 30°C and pH =8.0. As
213 illustrated in Fig. 7, Percentage (%) removal of Bi³⁺ has been found to be higher at lower

214 concentration of bismuth solution (30 mg L⁻¹); a maximum bismuth removal of 97.60% has been
 215 obtained for *Streptomyces rimosus* biomass.

216

217 **3.8. Determination of biosorption isotherms**

218 Biosorption isotherms best describe the equilibrium behavior. An isotherm is a plot of amount of
 219 solute adsorbed per unit amount of adsorbent against the corresponding equilibrium concentration in
 220 the solution phase keeping temperature constant. Vital conclusion can be drawn from these isotherms,
 221 which are useful in designing of biosorption systems.³⁸ In order to determine the biosorption
 222 isotherms, batch experiments were performed in aqueous solution with initial bismuth concentration
 223 ranging between 5 and 120 mgL⁻¹. This latter solution was prepared by using a stock solution.
 224 Experiments were conducted for each bacteria strain at pH = 8.0 under magnetic stirring. In any run,
 225 the pH was measured, controlled and corrected to pH= 8.0 with 0.1 mol L⁻¹ HCl and/or NaOH. After
 226 48 h, 2 mL of the suspension were taken and centrifuged at 9000 rpm. The supernatant was analyzed
 227 by atomic biosorption to compute the equilibrium bismuth concentration. The kinetics parameters in
 228 this study are given in Table 4. The experimental data had a better fit to the pseudo-second-order
 229 kinetic model than the pseudo-first-order kinetic model, based on the R² values presented in Table
 230 4. The theoretical q_{cal} values for bismuth were very close to the experimental q_{exp} values. This
 231 suggests that the biosorption data were well represented by pseudo-second-order kinetics. It was
 232 concluded that the biosorption mechanism involved chemisorptions between adsorbate and the
 233 adsorbent.³⁹

234

235 *3.8.1. Langmuir isotherm*

236 Langmuir isotherm is obtained from a kinetic derivation of equilibrium between adsorbed and
 237 desorbed molecules.⁴⁰ This gives

238

$$239 \frac{C_e}{q_e} = \frac{1}{Q_m K_L} + \frac{C_e}{Q_m} \quad (3)$$

240

241 where q_e (mg g⁻¹) is the equilibrium biosorption capacity denoted as adsorbent mass per weight unit
 242 of solid adsorbate, C_e (mg L⁻¹) is the equilibrium adsorbate concentration remained in solution and
 243 Q_m (mg g⁻¹) and K_L are the biosorption equilibrium constant related to the binding energy. One of
 244 the major characteristics of this model is the dimensionless separation factor, R_L = 1/(1 + K_LC₀) that
 245 points out whether the biosorption process is favorable (0 < R_L < 1), unfavorable (R_L > 1), linear (R_L =
 246 1) or irreversible (R_L = 0). The model parameters were obtained from the linear relation between
 247 C_e/q_e and C_e (Table 4).⁴¹

248

249 *3.8.2. Freundlich isotherm*

250 This isotherm is derived from empirical consideration and expressed as

251

$$252 \ln q_e = \ln K_F + \left(\frac{1}{n}\right) \ln C_e \quad (4)$$

253

254 Where, K_F is the Freundlich constant, which is a measure of biosorption capacity or fundamental
255 effectiveness of the adsorbent. It is directly related to the standard free energy change, and n is an
256 empirical constant related to heterogeneity of the adsorbent surface. Thus a plot between $\log q_e$ and
257 $\log C_e$ is a straight line. Values of K_F and $1/n$ are calculated from plotting of graph between $\log q_e$ and
258 $\log C_e$. A high K_F and high 'n' values are indicative of high biosorption throughout the concentration
259 range. A low K_F and high 'n' values indicate low biosorption throughout the studied concentration
260 range. A low 'n' value indicates high biosorption at strong solute concentration.

261

262 **3.9. Determination of biosorption kinetics**

263 The Lagergren's first-order and pseudo-second-order models were used to test biosorption kinetics
264 data to investigate the mechanism of biosorption. The Lagergren pseudo-first-order model (Eq. (5))
265 and pseudo-second-order model (Eq. (6)) equations are:^{42, 43}

266

$$267 \ln \left(\frac{q_e}{q_t} \right) = k_1 (q_e - q_t) + \ln q_e \quad (5)$$

268

$$269 \frac{t}{q_t} = 1/(k_2 q_e^2) + t/q_e \quad (6)$$

270

271 Where q_e and q_t are the amounts of bismuth adsorbed by the adsorbent at equilibrium and at time t
272 (mg g^{-1}), respectively; k_1 and k_2 are the pseudo-first-order and pseudo-second-order constants (min^{-1}),
273 respectively; and t is the biosorption time. The kinetics parameters in the single and binary
274 systems are given in Table 5. The experimental data had a better fit to the pseudo-second-order
275 kinetic model than the pseudo-first-order kinetic model, based on the R^2 values presented in Table
276 5. The theoretical q_{cal} values for bismuth biosorption were very close to the experimental q_{exp}
277 values. This suggests that the biosorption data were well represented by pseudo-second-order
278 kinetics. It was concluded that the biosorption behaviors of bismuth were better described by the
279 pseudo-second-order kinetic model, which suggests that the biosorption mechanism involved
280 chemisorptions between adsorbate and the adsorbent.^{39, 44}

281

282 **4. Conclusion**

283 A bacterial biosorbent was an effective biosorbent for removal of Bi^{3+} from water solution. Response
284 surface methodology as less labor intensive and time-consuming approaches was used to optimize

285 effect of variables. RSM approach based on BBD efficiency was judged according to fitness of
286 polynomial equation, the model adequacy, lack-of-fit (LOF), *P-values*, *F-values* and subsequent
287 replication at optimum point suggested by model. The optimum biosorption conditions were
288 determined as initial pH 8.0; 30 °C; 48 h and initial concentration of 30 mg L⁻¹ Bi³⁺. The FTIR
289 spectroscopic analysis confirmed that biosorbent functional groups on the biosorbent are main reactive
290 sites to accomplish biosorption. It was also found that the biosorption process followed the Langmuir
291 isotherm model with the highest value of correlation coefficients (>0.980) and the biosorption capacity
292 being estimated to be 38.91 mg g⁻¹. The pseudo-first order and pseudo-second order models were
293 applied to the experimental data in order to kinetically describe the removal mechanism of bismuth,
294 with the second one showing the best fit with the experimental kinetic biosorption data ($R^2 = 0.999$).

295

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299

300

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Table 1. Matrix for the BBD.

				Levels				
Factors				Low (-1)	Central (0)	High (+1)	- α	+ α
X ₁ :pH				6.00	7.00	8.00	5.00	9.00
X ₂ : temperature (°C)				20.00	25.00	30.00	15.00	35.00
X ₃ : Bi ³⁺ concentration (mg L ⁻¹)				10.00	15.00	20.00	5.00	25.00
Factors				R% Bi ³⁺				
Run	X ₁	X ₂	X ₃	Actual ^a	Predicted ^b			
1 (F)	9.00	30.00	20.00	24.60	23.96			
2 (F)	7.00	30.00	20.00	52.50	43.70			
3 (F)	8.00	30.00	30.00	97.70	46.93			
4 (F)	9.00	30.00	40.00	17.50	64.30			
5 (F)	9.00	25.00	30.00	7.400	71.63			
6 (F)	9.00	35.00	30.00	5.600	57.50			
7 (F)	8.00	35.00	40.00	12.40	83.63			
8 (F)	8.00	25.00	20.00	43.70	74.79			
9 (F)	7.00	35.00	30.00	11.50	16.68			
10 (F)	8.00	35.00	20.00	18.90	44.70			
11 (F)	7.00	30.00	40.00	31.60	22.35			
12 (F)	8.00	30.00	30.00	97.40	74.30			
13 (F)	8.00	30.00	30.00	97.60	53.25			
14 (F)	8.00	25.00	40.00	21.30	70.13			
15 (F)	7.00	25.00	30.00	42.60	65.10			

432 ^a Experimental values of response.

433 ^b Predicted values of response by RSM proposed model.

434 F: Factorial point

435 A: Axial point

436 C: Center point

437 **Table. 2.** Analysis of variance (ANOVA) for BBD.

Factor	SS ^a	Df ^b	MS ^c	F-value	P-value	Status
Model	15530	9	1725	26200	< 0.0001	*
X ₁	863.2	1	863.2	13110	< 0.0001	*
X ₂	554.4	1	554.4	8422	< 0.0001	*
X ₃	404.7	1	404.7	6147	< 0.0001	*
X ₁ X ₂	214.6	1	214.6	3260	< 0.0001	*
X ₁ X ₃	47.61	1	47.61	723.2	< 0.0001	*
X ₂ X ₃	63.2	1	63.2	960	< 0.0001	*
X ₁ ²	4962	1	4962	75370	< 0.0001	*
X ₂ ²	7192	1	7192	109200	< 0.0001	*
X ₃ ²	3182	1	3182	48340	< 0.0001	*
Residual	0.3292	5	0.06583			
Lack-of-Fit	0.2825	3	0.09417	4.036	0.2049	**
Pure Error	0.04667	2	0.02333			
Total	15530	14				

438 ^aSequential sums of squares439 ^bDegrees of freedom440 ^cmean sums of squares

441 *significant

442 **not significant

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472 **Table. 3.**FTIR Spectra analysis

Before biosorption (cm ⁻¹)	After biosorption (cm ⁻¹)	Functional groups
3430.28	3442.1	O-H, N-H
2920.2	2920.2	C-H
2845.3	1652.7	C-H
1625.4	1646.3	-COO, C-O
1401.2	1401.2	COO-
1118.21	1046.45	-C-O, C-N

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515 **Table. 4.** Isotherm constant parameters and correlation coefficients calculated for the biosorption of
 516 bismuth onto *Streptomyces rimosus*.

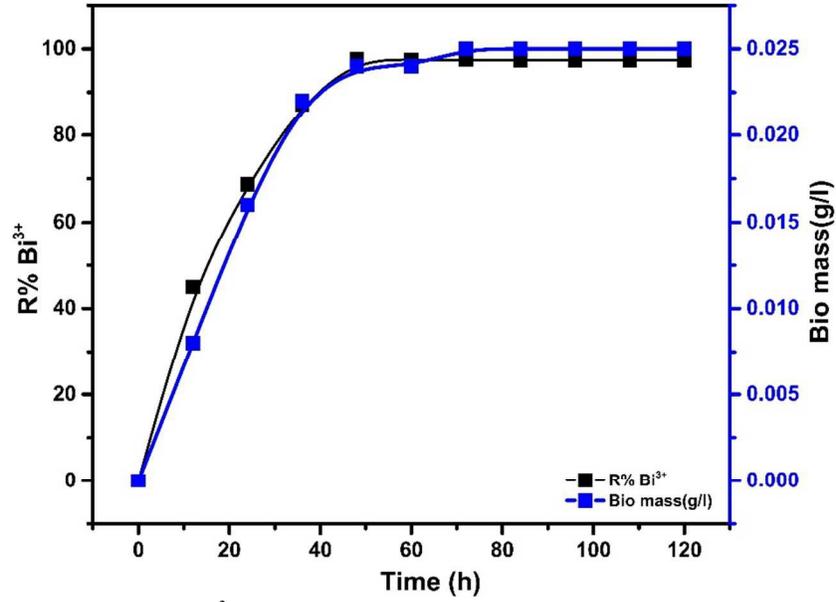
Isotherm	Parameters	Bi ³⁺
Langmuir	Q _m (mg.g ⁻¹)	38.93
	K _L (L mg ⁻¹)	1.01
	R ²	0.9944
	R _L	0.047-0.012
Freundlich	1/n	0.1266
	K _F (L mg ⁻¹)	4.037
	R ²	0.8009

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557 **Table 5.** Kinetic parameters for the biosorption of bismuth using *streptomyces rimosus*.

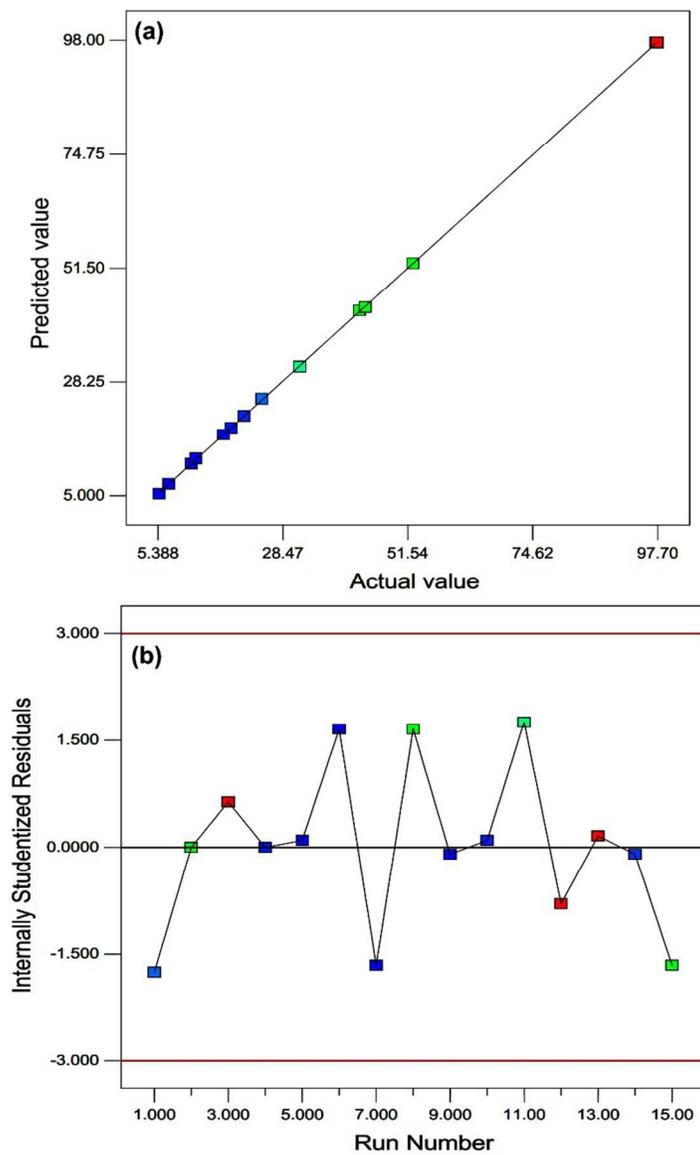
Model	Parameters	Values
Pseudo- First-order- kinetic	k_1 (min^{-1})	0.1566
	q_e (calc) (mg g^{-1})	4.431
	R^2	0.8282
Pseudo-second-order-kinetic	k_2 (min^{-1})	0.00047
	q_e (calc) (mg g^{-1})	13.489
	R^2	0.9999
	h ($\text{mg g}^{-1}\text{min}^{-1}$)	0.0855
Experimental Data	q_{eq} (exp) (mg g^{-1})	12.837

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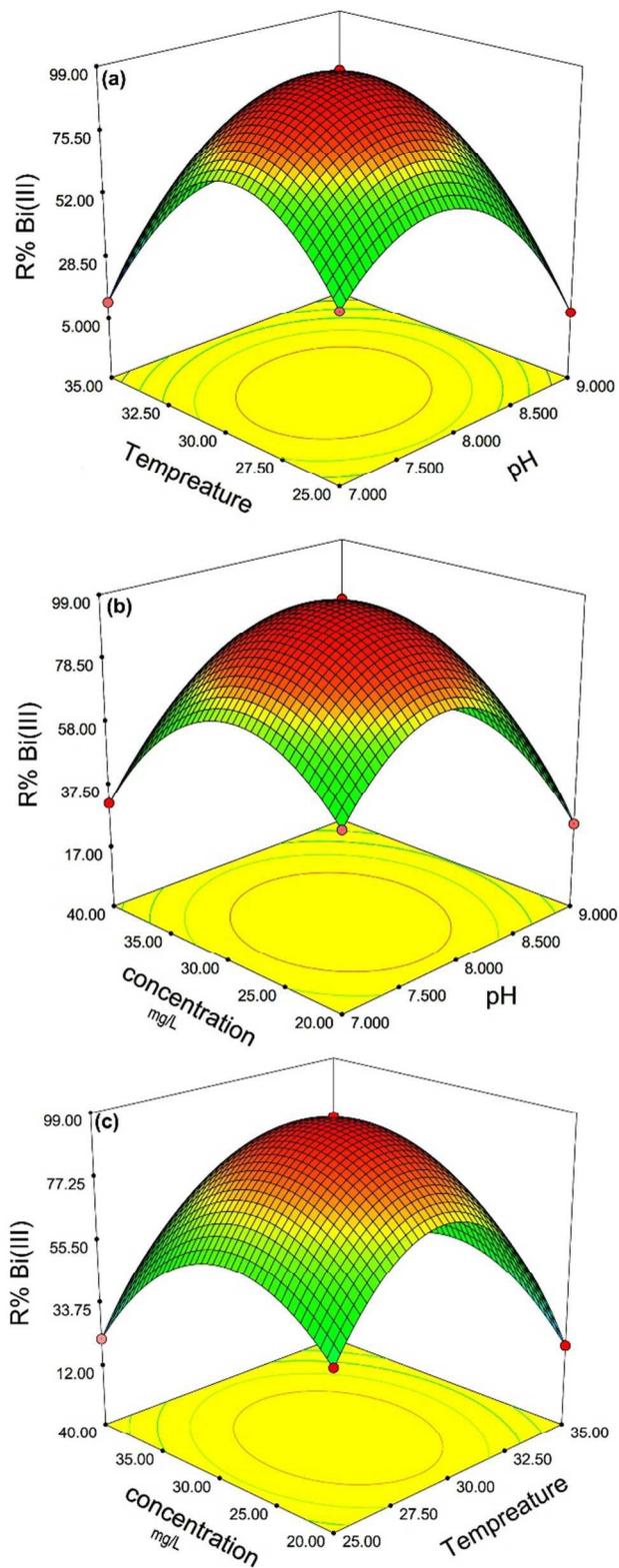


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587 **Fig. 1.** Effect of contact time on Bi^{3+} biosorption on *streptomyces rimosus* ($\text{pH}=5.5, T=25\text{ }^\circ\text{C}, C_0=30\text{ mg}$
588 L^{-1}).

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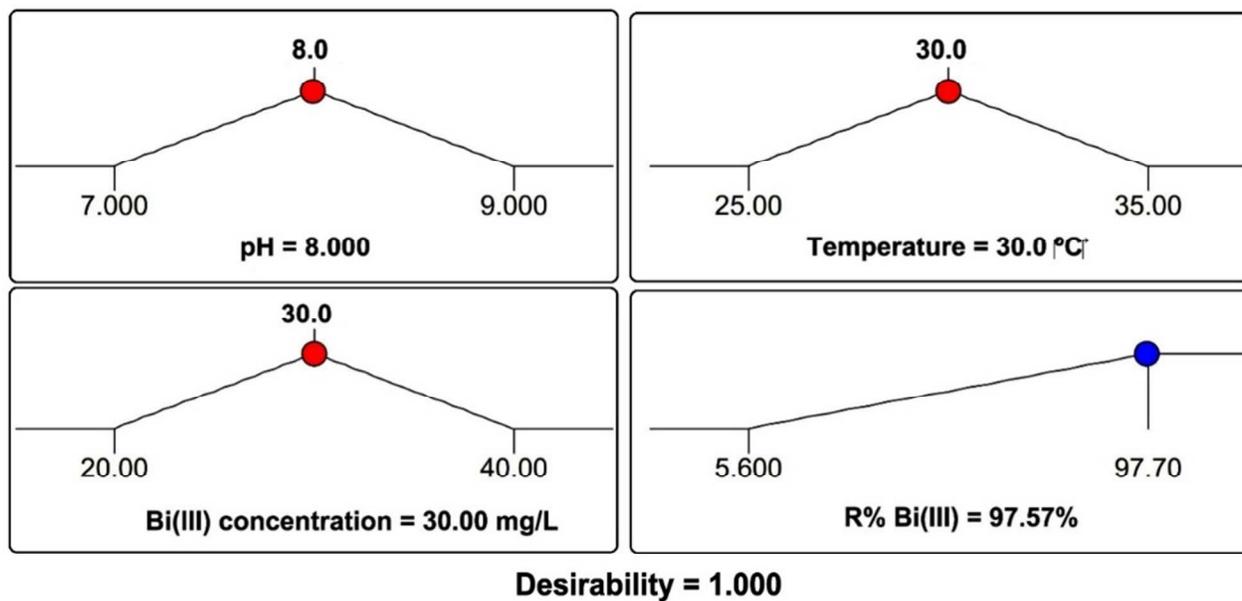


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621 **Fig. 2.**a) Correlation of actual and predicted percent biosorption and b) the studentized residuals vs. run
622 number plot for biosorption of Bi^{3+} .



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 624 **Fig. 3.** 3D surface plots of the biosorption Bi^{3+} ions versus two independent factors: (a) pH-temperature,
 625 (B) initial ion concentration – pH and (C) initial ion concentration - temperature.
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Fig. 4. Desirability ramp for numerical optimization of three goals, namely the pH, temperature, initial Bi^{3+} concentration and Bi^{3+} removal.

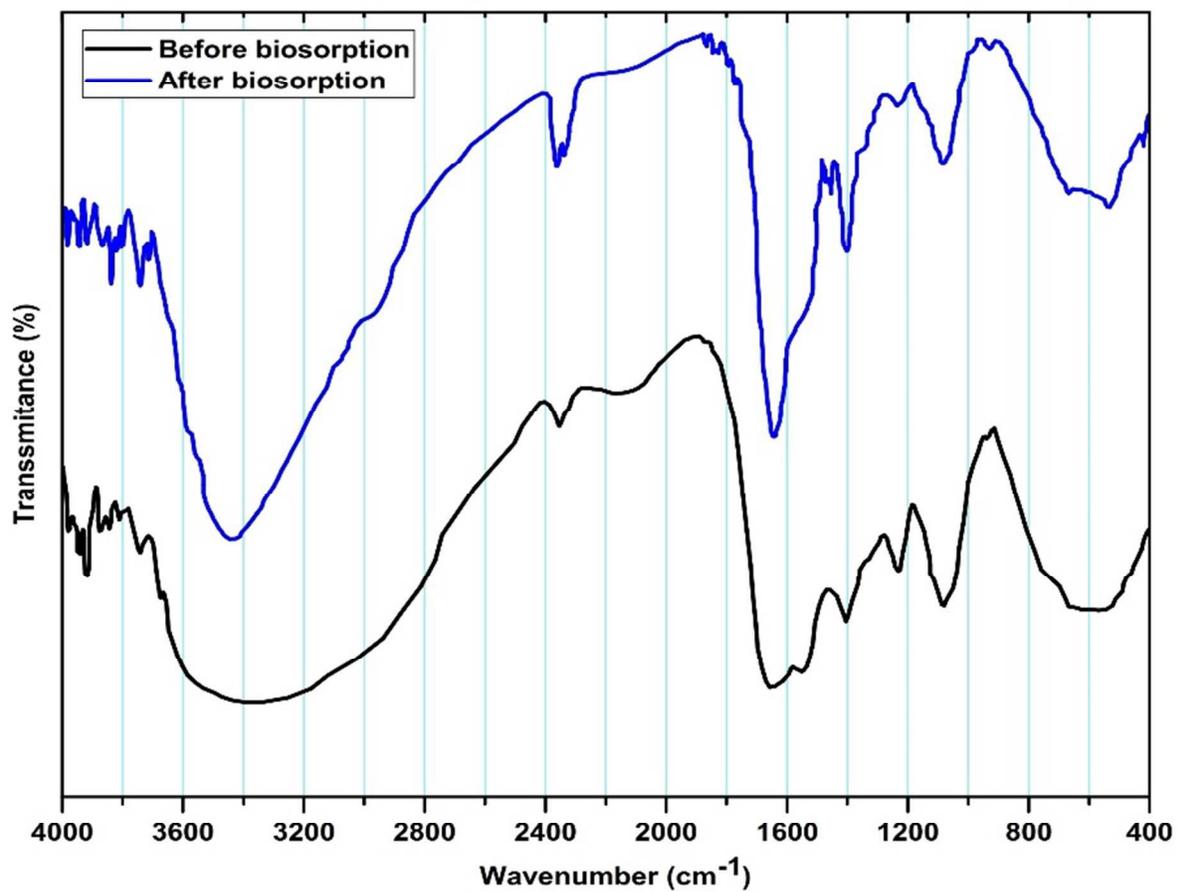
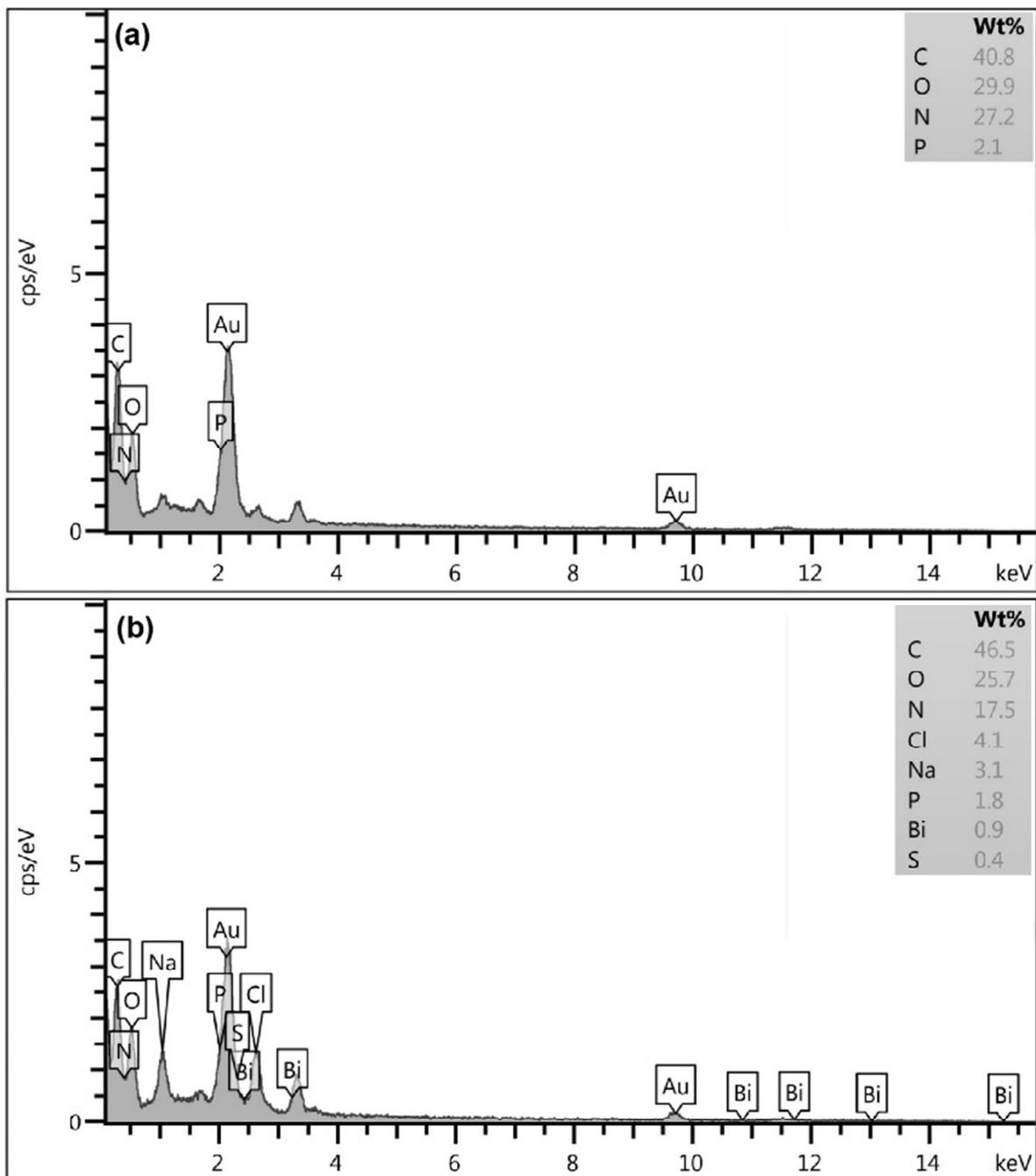
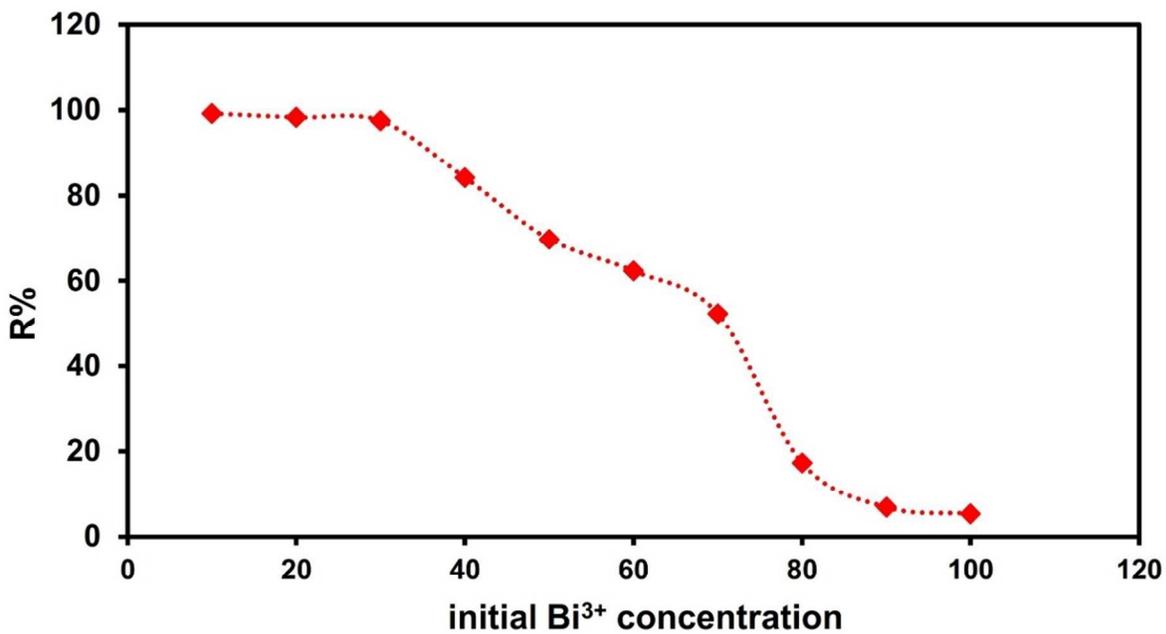
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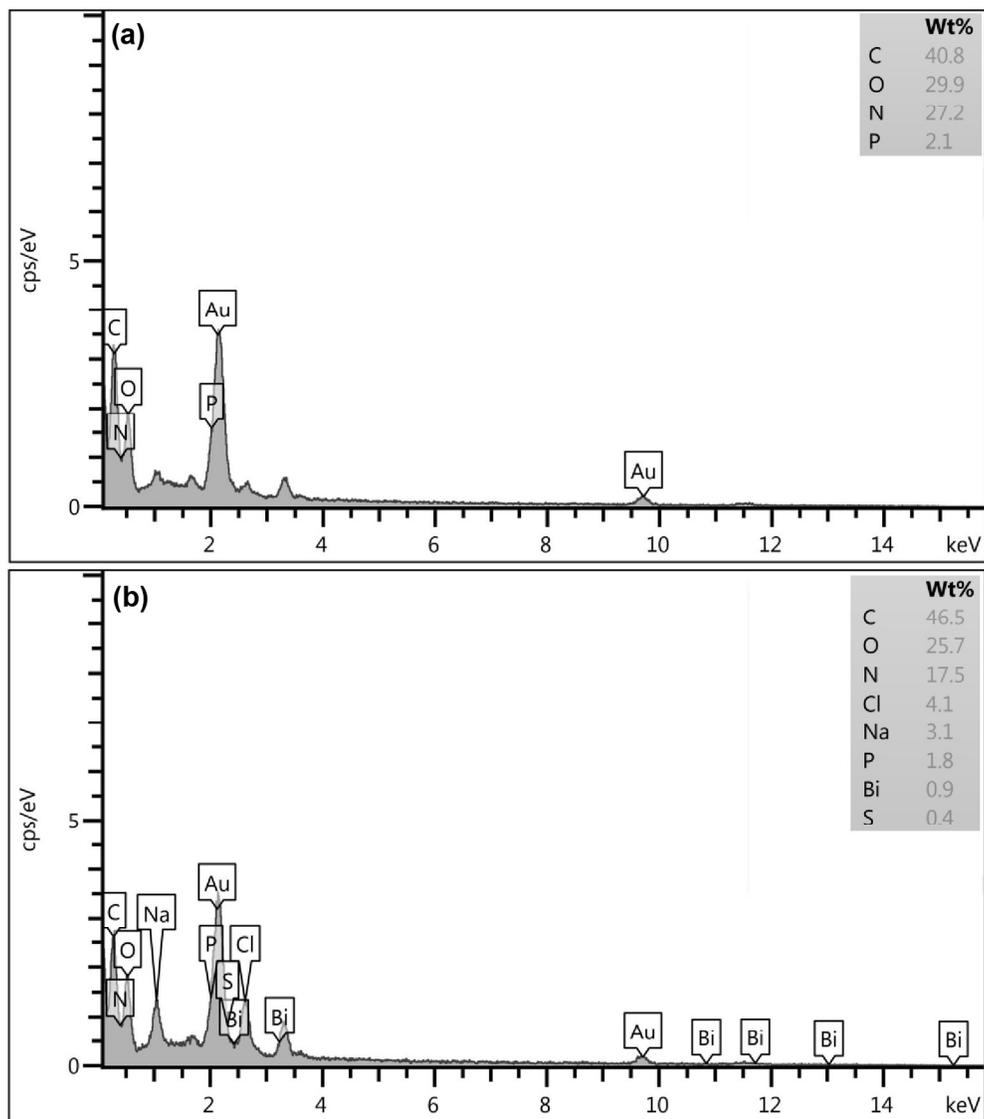
Fig. 5. FTIR spectra of *streptomyces rimosus* for biosorption of Bi³⁺ ions: before and after biosorption.



682
 683 **Fig. 6.** EDAX spectra of *streptomyces rimosus*: (a) in the absence of bismuth and (b) after bismuth
 684 biosorption ($C_0 = 30 \text{ mg L}^{-1}$).
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694 **Fig. 7.** Influence of initial Bi³⁺ concentration on removal efficiency and biosorption capacity of
695 *streptomyces rimosus* (contact time =48 h, temperature =30 °C and pH =8.0).
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147x167mm (300 x 300 DPI)