

Removal of Hg(II) from Aqueous Solution by *Bacillus subtilis* ATCC 6051 (B1)

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Abstract

Hg (II) has been as one of the most toxic metals on health hazards. The biosorption of toxic metals from aqueous solution by biomass is effective as an alternative method in recent years. Therefore, the biosorption of Hg(II) was examined in view of solution pH, initial concentration of Hg(II), contact time and reaction temperature by using *Bacillus subtilis* ATCC 6051(B1). Concentration of Hg (II) was measured by ICP-MS. FT-IR was employed for the characterization of bacterial surface. pH of 4.5 was found as the best acidity of aqueous samples. The results from kinetic studies suggested that Hg(II) bio adsorption was best represented by the pseudo second order equation. Results from equilibrium data fitted to Langmuir isotherm model. While ΔH° and ΔS° was obtained as 76.01 kJ/mol and 239, 11 kJ/mol respectively, ΔG° had a low value (4.76 kJ/mol). This study demonstrated that *Bacillus subtilis* having low cost can be used as an effective biosorbent for Hg(II) removal from aqueous solutions.

Keywords: Biosorbent; Adsorption; Hg(II); Bioaccumulation; Anthropogenic

Introduction

Mercury (Hg) is a liquid metal with flow properties in the elements and the compounds are also present in the form of Hg_2^{2+} and Hg^{2+} . The Hg (I) form is not free in aqueous solutions but the Hg(II) form is usually found in aqueous solutions and foods. Metallic Hg becomes in the vapor form is very dangerous and poisonous for livings [1-4]. Anthropogenic activities are the main reason of environmental Hg pollution. Bioaccumulation in environment/food chain, and non-degradable nature should be considered while evaluating its toxicity [5,6]. Due to the developing fisheries activities, toxic metals, especially mercury, are taken and these pollutants harm the health over time [7-10]. According to WHO, the major effects on human health of mercury poisoning are renal disturbances, neurological and impairment of pulmonary functions. It covers a wide range of habitat, such as forests, marshes, and agricultural land [11]. Nature also receives toxic metals from developing anthropogenic activities and acts as a temporal adsorbent for these pollutants. For example, industrial wastewater, floods into the coastal areas and pours into the rivers [12,13]. Moreover, elemental mercury is spreading as a mercury vapour that affects the farthest regions of the earth. Even uncontaminated coastal sediments had a mercury concentration of 0.39 to 1.62 $\mu g g^{-1}$ [14-16]. For this reason, green technologies such as biosorption are required to remove the pollutant from environmental samples [17-22] instead of classical methods such as ion exchange, and precipitation. For this reason, working with microorganisms is promising for removal of metals from wastewater; mining and industrial waste [18-21]. Compared to some microorganisms such as fungi, algae and yeast, the toxic metal capacity has proved to be the highest owing to adsorption cell wall of algae [23-26]. A number of microalgal strains such as the green algae species *Scenedesmus* sp., *Chlorococcum* sp., *Chlorella vulgaris* and the blue-green algae species *Phormidium molle*, *Aphanothece halophytica*, *Tolypothrix tenuis*, *Stigonema* sp., *Chroococcus paris*, *Lyngbya spiralis* and *Spirulina*, which are potentially suitable for Hg(II) and the other toxic elements removal in aqueous solution, were used in different studies and showed varying removal efficiencies, binding constants (K_b) and maximum adsorption capacities (q_{max}) [27-34]. Therefore,

biosorption of Hg^{2+} from aqueous solutions was investigated in details by *Bacillus subtilis* ATCC 6051.

Materials and Methods

Instrumentation

Bruker Aurora M-90 ICP-MS (Inductively Coupled Plasma-Mass Spectrometer) was used to measure of Hg concentrations. Millipore Milli-Q (Direct-Q UV3, USA) was used to obtain ultra pure water. pH measurements, agitation and precipitation were made using a pH meter (Hanna HI-2211700, USA), shaker (Nuve) controlled temperature and centrifuge (ALC-4235A) models. The scanning electron microscopy (SEM/EDX, EVO 40 LEQ model) was used for measurement of image the bacteria size. TGA and DTA (Shimadzu TGA-50 series model) studies were carried out in the between temperature range 20-750°C and the infrared spectrums was used Mattson-1000 model FT-IR spectrophotometer.

Reagents and standard solutions

In this study; HNO_3 , HCl, NaOH, Nitrate salt of Hg(II) are used analytical grade obtained from Merck, Germany. An aqueous stock solution $1004 \pm 4 \mu g/ml$ of Hg(II) (Plasma CAL, SCP Science, USA) was used in all experimental. Standard solution for ICP-MS was obtained from $1004 \pm 4 \mu g/ml$ stock solutions. Required amounts of 0.1 M NaOH and HCl were used for pH adjustments.

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Analytical sensitive and accuracy of the method for ICP-MS

For the analytical sensitive and accuracy of Hg(II) elements was calculated using calibration curves which were prepared from the 1004 ± 4 µg/ml stock solution (Plasma CAL, SCP Science, USA). Linearity of analytical curves was found higher 0.999. The limit of detection (LOD) and limit of quantification (LOQ) of Hg(II) was calculated by analytical curves performed with analysis 10 times average of a blank solution spiked with the element at a level of lower concentration. The LOD and LOQ were calculated from the standard deviation (S_d) and equation (LOD=X_{avr.}+3S_d, LOQ=X_{avr.}+10S_d) The certified reference material (CRM) solution for wastewater (ERA A water Company Certificate of Analysis Lot No: P232740A) was used to validation of the method. The samples were analysed in triplicate by ICP-MS [35,36]. The analytical results were in good agreement with the certified values (Table 1).

Biosorbent preparation

Bacillus subtilis ATCC 6051(B1) were used in these experiments. The morphological characterization of the organism was also done with the bacterial culture such as gram and endospore staining. To produce the biomass for biosorption experiments, each microorganism was inoculated to 1 L of liquid Nutrient Broth and it was left to incubation in a shaker at 37°C for 24 hours. After centrifugation at 7000 rpm for 15 min, it was washed twice. Final product was dried at 65°C for 24 h and sieved to under 180 µm size and protected in sterile sample bottles for use in biosorption studies [37,38].

Results and Discussion

In this study, the effect of metal concentration, biosorbent mass, retention time, temperature and pH on the adsorption of Hg(II) were investigated. TGA/DTA, FT-IR and SEM/EDAX were used for surface characterization. The data obtained from results were calculated for the Langmuir-Freundlich adsorption isotherms and biosorption kinetic models (Pseudo first-second order). The equilibrium adsorption capacity (q_e) in all experiments was calculated using equation (1) from differences initial (C₀) and equilibrium (C_e) concentration [19,26,39,40]. The equilibrium sorption capacity (q_e) is the amount of metal ion sorbet at equilibrium (mg/g), C₀ initial concentration of Hg(II), C_e equilibrium concentration, V volume of solution Hg(II), m biomass dose and it can be expressed as follows:

$$qe = \frac{(co - ce).v}{m} \tag{1}$$

The Effect of Experimental Parameters on Adsorption Capacity

Biosorbent mass and pH

50 ml of 50 mg/L Hg (II) solution was conditioned to different pH (pH from 1 to 8) with 0.1 N NaOH and HCl. Suspensions of bacterial cells were contacted with pH adjusted Hg(II) solution. The solution was incubated at 25°C in a shaking incubator (150 rpm) for 60 min. The equilibrium adsorption capacity (q_e) was calculated from equation (1) and about 72.14 mg/g was observed at pH>4.5 (Figure 1) for initial concentration of Hg(II) (50 mg/L). The effect of biosorbent mass on the biosorption of Hg (II) by *Bacillus subtilis* ATCC 6031 was studied with the range of the biosorbent from 10 to 50 mg with initial metal

concentration of 50 mg/L in 50 ml volume and optimal pH 4.5. The equilibrium adsorption capacity (q_e) was calculated from equation (1) and increased biosorbent mass was found to have a negative effect on decreased Hg (II) uptake. It can be observed from Figure 2 that the uptake of Hg (II) with decrease from 143.22 to 35.18 mg/g by increasing the biosorbent mass from 10 to 50 mg. The increase in the biosorption dose can be attributed to the availability of more adsorption sites. The decrease in adsorption capacity may be due to the reduction in the rate of metal ions bound to the adsorbent surface [41].

Temperature and biosorption isotherm

The effect of temperature on mercury biosorption by the *Bacillus subtilis* ATCC 6031, temperature was varied at 298, 308 and 318 K as shown in Figure 3. The experiments were performed with an initial Hg(II) concentration of 5,10,15,20,25 mg/L, the biomass concentration of 5 g/L at the optimum pH of 4.5 and the contact time 60 min. It was observed that uptake of Hg increased with increasing in temperature from 298 to 318 K. However, ambient temperature was selected as the best due to no significantly differences.

Freundlich and Langmuir isotherms

The adsorption isotherm models are generally based on the isotherm equations Freundlich and Langmuir [34,39,40]. Correlation coefficient (R²) is the indicator of fitting to any model. The Langmuir equation is given as follows:

$$qe = Q_{max} \cdot \frac{b.Ce}{1 + Ce} \rightarrow \frac{Ce}{Qe} = \frac{1}{Q_{max}} \cdot Ce \tag{2}$$

Where q_e is the equilibrium metal concentration on the biosorbent (mg/g), C_e is the equilibrium metal concentration in solution (mg/L), Q_{max} is the monolayer capacity of the biosorbent (mg/g) and b is the Langmuir constant. The Langmuir constants for biosorbents were calculated correlation coefficient (R²), Q_{max} and b constants from equation (2) (Table 2). The Freundlich isotherm model equation [16,26,27,41,42] is expressed as follows:

$$qe = k_f \cdot C_e^{1/n} \rightarrow \log qe = \log k + \frac{1}{n} \log Ce \tag{3}$$

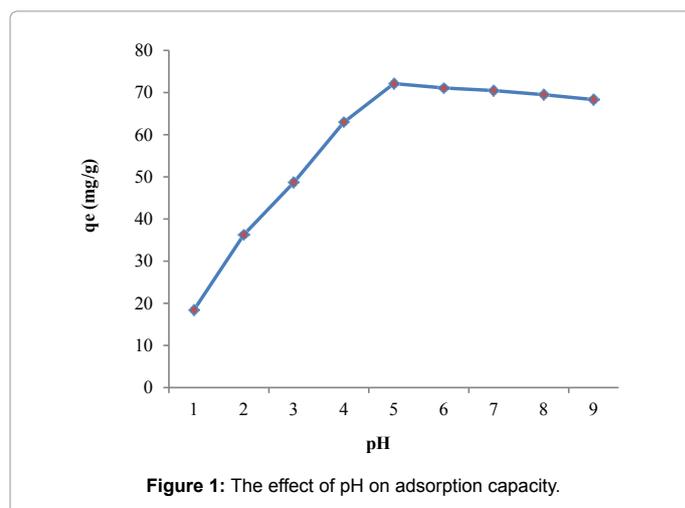


Figure 1: The effect of pH on adsorption capacity.

Atomic spectroscopy	LOD (µg/ml)	LOQ (µg/ml)	Certified values (µg/L)	Confidence measured (µg/L)	Profession testing (µg/L)
ICP-MS ²⁰² Hg	0.4020	1.3390	631 ± 0.046	705 ± 0.062	536-726

Table 1: The analytical sensitivity and accuracy for ICP-MS.

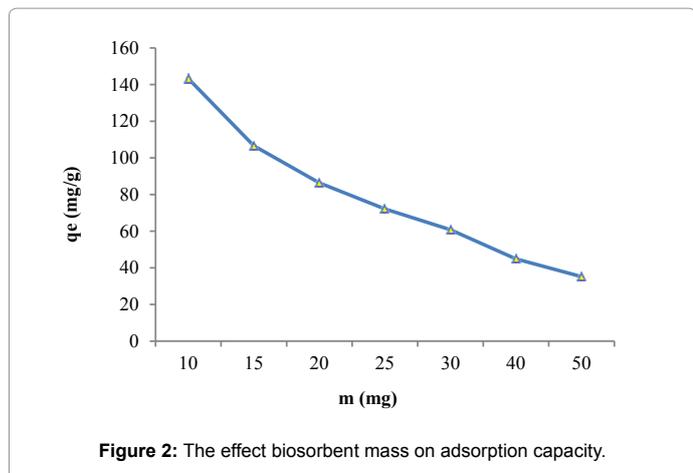


Figure 2: The effect biosorbent mass on adsorption capacity.

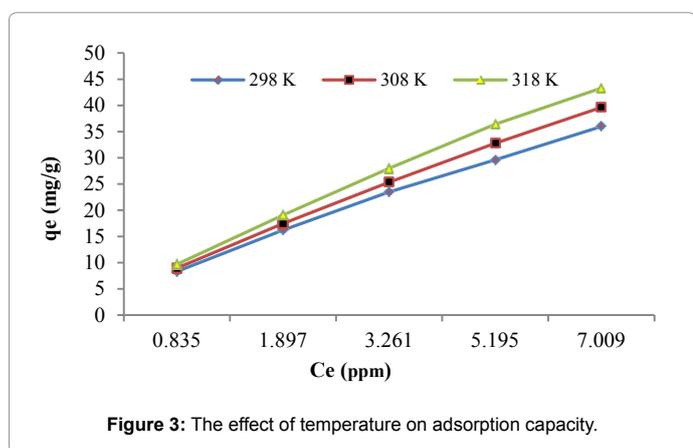


Figure 3: The effect of temperature on adsorption capacity.

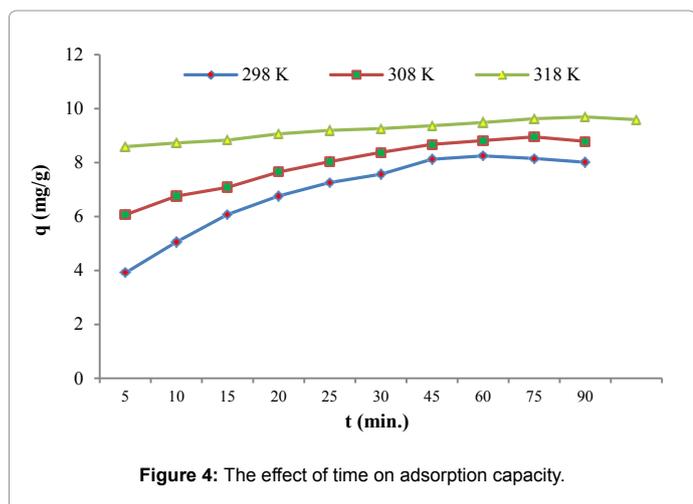


Figure 4: The effect of time on adsorption capacity.

Where K_F is the Freundlich adsorption capacity, C_e is the equilibrium metal concentration in solution (mg/L), n is the Freundlich constant. The Freundlich constant for biosorbents (B1) was calculated correlation coefficient (R^2), K_F and n constants from equation (3) (Table 2).

The experimental data on biosorbents (B1) of Hg(II) show that the isotherms correlation coefficient (R^2) are agreement with the Langmuir type, as can be seen from the magnitude of the R^2 values in the Table 2. As seen in the table, monolayer biosorption capacities (Q_m) were found to vary between 55 and 67 mg/g depending to the temperature.

Contact time and biosorption kinetics

Effect of time was investigated in the range of 5-75 min at three different temperatures. As shown in the Figure 4, rapid adsorption was observed in 10 min. of contact time with increasing of temperature. After that the rate reaction continues in till reach a constant value of Hg(II) concentration within 75 min. After this time, there was a very small increase in Hg involvement, as the biosorbent had little active surface area on the cell wall.

The reaction mechanisms of biosorption process were used the pseudo-second-order and pseudo-first-order to interpret the experimental data. Lagergren equation was also employed to find the rate constant. The pseudo-first and second-order equations can be expressed as follows:

The pseudo-first-order equation:

$$\frac{dq_t}{dt} = k_1(q_t - q_e) \rightarrow \log \frac{q_e - q_t}{q_e - q_0} = -\frac{k_1}{2,303} t \rightarrow \log(q_e - q_t) = \log q_e - \frac{k_1}{2,303} t \quad (4)$$

The pseudo second-order equation:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \rightarrow \frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \rightarrow \frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (5)$$

where q_e and q_t are the amount of Hg(II) biosorbed (mg/g) in equilibrium at any time (t), k_1 and k_2 is the rate constant for the pseudo-first and second-order kinetics (min^{-1}). The best fit of each kinetics model for biosorbent (B1) was evaluated in terms of correlation coefficient (R^2). To determination of the pseudo-first and second equation constant for biosorbent in different time(min.) and temperature ($^{\circ}\text{C}$) were calculated correlation coefficient (R^2), k_1 (pf) and k_2 (ps) constant from equation (4) and equation (5) (Table 3).

The results were summarized in Table 3. It could be easily said from the results that pseudo second order model for valid. The second-order kinetic rate constants (k_{ps}) decrease with the increase of the initial Hg(II) concentration and increases with increasing temperature [26,41,42]. This may be due to Hg(II) ions present at high concentrations in the solution. They compete with each other and cause a delay in reaching the equilibrium of low k_{ps} values. The high applicability of the second-order equation of Hg (II) ions to the various adsorbents was also determined.

Characterization of Biosorbent

FT-IR spectral studies

T (K)	Langmuir constants			Freundlich constants		
	Q_m (mg g ⁻¹)	b (L mg ⁻¹)	R^2	k (L g ⁻¹)	1/n	R^2
298	66.67	0.172	0.992	9.247	0.680	0.991
308	66.67	0.288	0.993	14.190	0.648	0.994
318	55.56	1.200	0.995	26.122	0.493	0.984

Table 2: The isotherm constants of Langmuir and Freundlich for biosorbents (B1) in different temperature.

T (K)	Pseudo first order kinetic model			Pseudo second order kinetic model		
	qe (mg g ⁻¹)	kpf (min ⁻¹)	R ²	qe (mg g ⁻¹)	kps (g mg ⁻¹ min ⁻¹)	R ²
298	7.852	0.0852	0.986	79.142	0.0126	0.998
308	4.121	0.0598	0.988	35.549	0.0281	0.997
318	1.271	0.0322	0.956	8.2540	0.1211	0.999

Table 3: The kinetic models in different temperature for biosorbent (B1).

Frequency (cm ⁻¹)		Functional group	Frequency (cm ⁻¹)		Functional group
B1	B1-Hg		B1	B1-Hg	
3263.99	3279.86	-OH, -NH	1445.84	1443.09	-CH
3060.43	3060.43	-	1395.82	1380.00	COO ⁻ , C-N
2926.95	2927.03	-CH	1322.38	-	-
1630.60	1637.12	Amide I (C=O)	1219.00	1209.47	C-N, P-O-C
1530.31	1523.57	Amide II (COO ⁻ , -C(=O)-NH-, C=O)	1040.41	1026.10	P-O

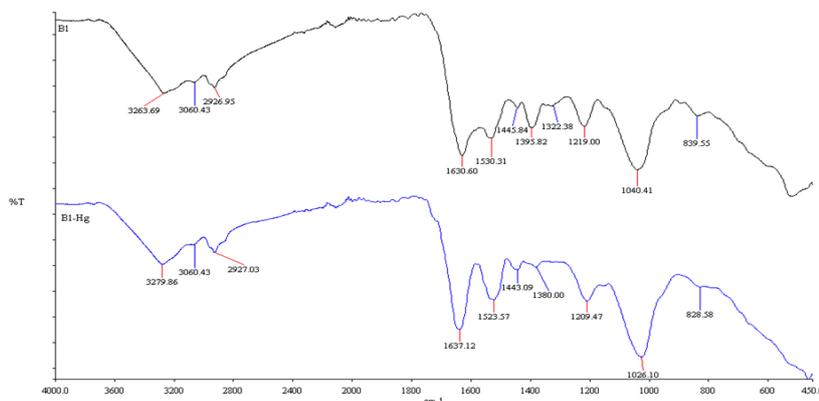


Figure 5: ATR-FTIR spectra of biosorbent before and after the biosorption of Hg.

The FT-IR spectra of B1 with and without Hg were presented in Figure 5. The peaks with respect to related functional groups were also discussed on Figure 5 [39,40].

As seen in Figure 5 and FT-IR spectrum, strong asymmetric stretching bands belonging to functional groups such as OH⁻, -NH, Amid (I, II), P=O, COO⁻, C-O-on surface B1 biosorbent were observed. After then the biosorbents was activated with Hg(II), it was found that there were some shifts in functional group bands. The spectra indicated that the Hg(II) ions was adsorbed by nature of functional groups. The spectra of *Bacillus subtilis* sp. obtained from ATCC 6051 are similar to nearly FTIR spectra used by other workers.

The thermal analysis (TGA/DTA) of biosorbent

The main purpose of the analyses with TGA are to examine the disruption processes of the biosorbents (B1) depending on the temperature [40,42]. In this study, the biosorbents were analysed at 25-1000°C with TGA and DTA data at a flow rate of 20 mL /min. (under N₂ purge, 10°C /min of temperature increase rate). The results are shown in the Figure 6.

According to the result of TGA and DTA data, the first mass loss of the adsorbents appears to be between 25 and 109°C due to physically adsorbed water on the surface of the adsorbents and at temperature

between 109-211°C is due to water trapped in the bacteria. The carbonation appears to occur between 211-343°C and they are burning in temperatures above 343°C. Therefore; TGA and DTA data show that the biosorbents (B1) can be used for adsorption studies up to 211°C and resistant to heat.

SEM/EDX analysis

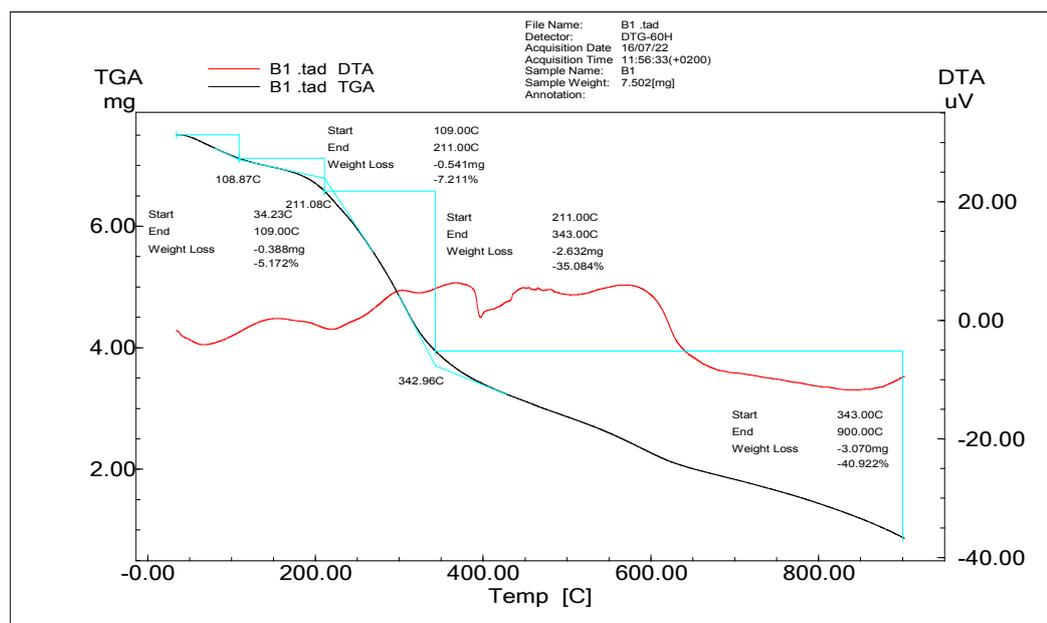
SEM image/EDX spectra obtained with high vacuum EVO-440 model device under 20 kV. Untreated and treated Hg (II) biosorption on *Bacillus subtilis* ATCC 6031 biomass are presented in Table 4. Figure 7 shows the presence of K, Na, Mg, P and S on the surface of *Bacillus subtilis* ATCC 6031 was confirmed by EDX. The EDX analysis in the biomass treated with Hg(II) ion shows the presence of mercury spectrum and intensity increase of Na spectrum, significant intensity decrease of K spectrum. From the results, it could be concluded that exchange of metal ions is an effective way of biosorption [39,42].

Recovery studies

In this study, the biosorbent B1 saturated with Hg were treated at different concentrations (0.01, 0.05 and 0.1 mol/L) with HCl and HNO₃ acid. The results showed that with 0.1M HNO₃ was removed in between 97-99% from the biosorbents. However, HCl was little effect on the recovery because of the formation precipitate HgCl₂ in

Biosorbent	Element/A. No.	Measured amount (wt. %)	Mass amount (wt. %)	Atomic ratio (%)	Error rate (%)
Untreated B1 Series	Na:11	0.50	4.33	6.26	0.1
	Mg:12	0.47	4.07	5.56	0.1
	P:15	4.71	40.81	43.75	0.2
	S:16	0.80	6.93	7.18	0.1
	K:19	5.06	43.84	37.24	0.2
Treated B1-Hg ²⁺ Series	Na:11	3.14	6.70	32.59	0.26
	Mg:12	0.86	1.84	8.44	0.10
	P:15	3.78	8.07	29.11	0.23
	S:16	1.03	2.20	7.66	0.10
	K:19	1.43	3.05	8.72	0.10
	Hg:80	36.59	78.13	43.51	2.05

Table 4: The spectrum rates of mineral untreated and treated for *Bacillus subtilis* (B1).



Starting and ending temperature (°C)	: 25-109	109-211	211-343	343-900
Weight loss (%)	: 5.17	7.21	35.38	40.92

Figure 6: The TGA and DTA data of biosorbent *Bacillus subtilis* (B1).

solution. The recovered bacteria were re-examined on the adsorption and observed to retain the adsorption capacities which these features proved that bacteria can reuse in the adsorption of the metals [26].

Conclusion

The *Bacillus subtilis* obtained from ATCC 6051(B1) were determined characteristics and adsorption capacities. As a result of

the studies carried out, it has been determined that it can be easily applied to removal toxic element Hg from wastewater and drinking water because of having good morphological characteristics, recovery, temperature and water resistance. The *Bacillus subtilis* for the removal of Hg(II) from the aqueous solution can be promising more effective as an alternative method chemical and physical processes because of its high metal adsorption capacity, easily obtained in large quantities,

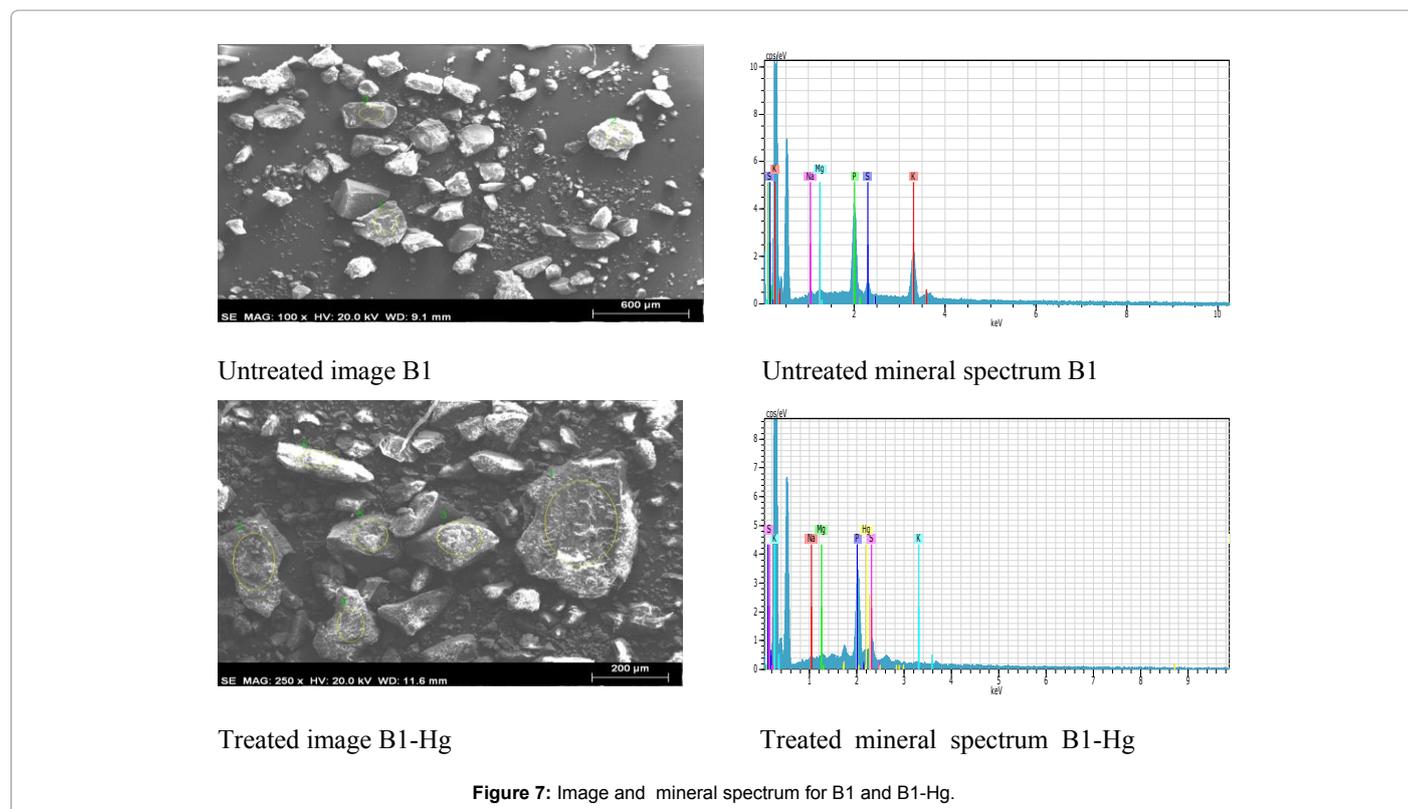


Figure 7: Image and mineral spectrum for B1 and B1-Hg.

high efficiency, water resistant, re-applicability, low cost and in dilute solution effluents.

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