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Environmental remediation of thorium(IV) from aqueous medium onto *Cellulosimicrobium cellulans* isolated from radioactive wastewater

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ABSTRACT

Cellulosimicrobium cellulans (*C. cellulans*) was isolated from radioactive wastes and identified by Biological examination. The investigation of the removal of thorium from aqueous solutions was carried out using the isolated living and dead *C. cellulans*. The biosorption of thorium was studied using different thorium ion concentrations. Electron microscopic examinations of both living and dead *C. cellulans* before and after biosorption of thorium ions were done to locate the sites of metal ion biosorption and to find the difference between living and dead bacterial cells. The obtained results showed that living and dead *C. cellulans* could sorp 151.94 and 220.56 mg/g, respectively. The kinetic behavior and biosorption isotherm were defined. These data kinetically followed the pseudo-second-order model and indicated a good fitness with the Langmuir model.

Keywords: Biosorption; *Cellulosimicrobium cellulans*; Thorium

1. Introduction

The releases of industrial and radioactive wastes to the environmental are a potential health hazard and contaminate the soil and groundwater. The imposition of stricter regulations increases the demand for innovative treatment technologies to remove metals from water [1]. The use of adsorbent of biological origin has emerged in the last decade as one of the most promising alternatives to conventional techniques [2–4]. High metal-binding capacities of several biological materials had been identified. Among the biosorbents, there are natural waste products such as chitosan [5–8], marine algae [9,10], bacteria [11,12], yeast, [13] and fungi and waste mycelia from fermentation [14,15]. The major advantages of biosorption over conventional treatment methods include: low cost, high efficiency, minimization of chemical and/or biological sludge, no additional nutrient requirement, regeneration of biosorbent, and possibility of metal recovery [16].

Biosorption process could involve several mechanisms such as ion exchange, physical adsorption, complexation, and precipitation [17]. Biosorption mechanisms can be divided into two types: metabolism dependent and non-metabolism dependent. Metabolism dependent is a slow process inclusive of transport across cell membrane and precipitation

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[18,19]. While non-metabolism dependent is a rapid process, inclusive of precipitation, physical adsorption, ion exchange, and complexation [20]. The process is classified as (i) extracellular accumulation/precipitation, (ii) cell surface sorption/precipitation, and (iii) intracellular accumulation [18,19].

Both living and dead micro-organisms such as algae, bacteria, fungal, and yeast were used as biosorbent materials for heavy metal biosorption [21,22]. The feasibility of using these micro-organisms as a biosorbent for metal removal was searched for, as it is cheap and abundant in nature [23–26]. In the literature, some micro-organisms are capable of removing heavy metals even at low concentration, in the range 1–100 mg/L [27,28]. The advantage of using living cells over dead cells as a biosorbent is that living cells work similarly as dead cells at low metal concentration and living cells were able to generate new cells through growth, which allowed more space for biosorption mechanism to occur [29]. Guo et al. [30] reported that living cells could adsorb metal ions rapidly and provide high degree of separation.

The ultimate goal of this study is to isolate living C. *cellulans* from radioactive wastewater for the removal of thorium(IV) from aqueous media. The effect of various parameters, such as contact time, metal ion concentration on the biosorption process of Th(IV) onto both living and dead *C. cellulans* will be clarified.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the present work including thorium chloride, sodium hydroxide, sodium chloride, hydrochloric acid, nutrient agar ,and nutrient broth medium were of analytical purity grade. The stock solution of Th(IV) was prepared and other concentrations were obtained by dilution.

2.2. Isolation of bacteria

The studied bacteria were isolated from wastewater collected from Inshas Nuclear Reactor. The wastewater sample was analyzed by High Purity Germanium (HPGe) detector in order to determine the radioactive isotope constitute in the water sample (the sample volume was 1,000 mL and then evaporated to 100 mL).

The isolation of bacteria was carried out using the streak plate method [31]. The isolated bacteria were identified according to the earlier reported method by Holt et al. [32]. Thereafter, identification of bacteria species was confirmed by Biological examination [33], which was carried out at the Microorganisms Identification and Biological Control Unit in the Agriculture Research Centre, Giza, Egypt. The isolates were identified as *C. cellulans*.

2.3. Production of biomass

The isolated bacteria were cultivated by removing a loopful of bacteria from the cultivated slant to test tubes containing 5 mL nutrient broth composed of 1 g of Lab Lemco powder, 2 g of yeast extract, 5 g of peptone, and 5 g of sodium chloride in one-liter bidistilled water. These test tubes were incubated for 24 h at 30°C. The obtained cultures were transferred to one-liter conical flasks containing one-third of their volume nutrient broth and then incubated at 30°C for 24 h in an orbital shaking incubator. The cultivated bacterial biomass was harvested by centrifugation and then washed several times with bidistilled water. The obtained bacterial biomass was considered as living cells. The dead *C. cellulans* cells were prepared by autoclaving for 20 min at 150°C, and 1.5 kgf/cm^2, the autoclaved cells of *C. cellulans* were centrifuged to remove excess water before use. The obtained bacterial biomass (living and dead) was saved in a refrigerator for use.

2.4. Characterization of the biosorbent

2.4.1. Zeta potential measurements

Zeta potential measurements were performed for *C. cellulans* cells. A 0.01 g of dead cells was mixed with 50 mL of 0.1 M KCl. The suspension was then adjusted to the specified pH and kept under stirring for 15 min. After conditioning, the suspension was left for another 15 min for settling before measurements which were performed using zeta sizer 2000 (Malvern Instruments Ltd., London, UK).

2.4.2. Estimation of Th(VI)

The concentration of Th(VI) was measured spectrophotometrically at $\lambda = 545$ nm (in distilled water) using Jenway Spectrophotometer 6405 UV/Vis. Calibration curves were constructed according to Marczenko [34].

2.5. Uptake experiments

2.5.1. Effect of biosorbent dose

The mass of the biosorbent (living and dead *C. cellulans*) was varied from 0.1 to 1.0 g the desired mass was placed in a flask with 100 mL of 444.08 mg/L Th

(IV) ion (2.0 mmol/L) aqueous solution of Th(VI) at pH 4.5. The mixture was shaken using a Vibromatic–384 shaker at 300 rpm and 25°C. The uptake of Th(VI) was calculated by determining the residual concentration of Th(VI) following the above method and according to the following equation:

$$q_e = \frac{(C_0 - C_e) \times 0.1}{m} (\text{mg/g}) \quad (1)$$

where q_e is the uptake of Th(VI) at equilibrium, C_0 and C_e are the concentrations of Th(VI) at initial and equilibrium, respectively, and m is the mass of the used biosorbent material.

2.5.2. Effect of time

Different concentrations of Th(IV) ion were prepared from stock solution. The pH of the solution was adjusted at 4.5 ± 0.1 using drops of diluted nitric acid, then 0.2 g of wet bacterial biomass was immersed in 100 mL of Th(IV) ion. The flasks were conditioned at 300 rpm for a certain contact time. Five milliliters of the solution mixture was taken for centrifugation, and then the residual concentration of thorium was estimated spectrophotometrically according to the above method [34]. The adsorbed amount of Th(IV) per unit weight of the biomass beads q_t (mg/g) at time t was calculated from the mass balance equation as:

$$q_t = \frac{\sum_{i=1}^{n} (C_{t(i-1)} - C_{ti}) V_{t(i-1)}}{m} \quad (2)$$

where C_{ti} (mg/L) is the measured concentration of the drawn sample number i at time t and C_{t0} = (C_0), V_{ti} (L) is the volume of the solution in the flask at sample number i and time t, and m is the mass of the wet bacterial biomass added into the flask. Blank experiments were performed at the same time, where 0.2 g of bacterial biomass was immersed in 100 mL of bidistilled water. Each data point was taken as the average of three measurements with standard deviation of 2 ± 0.5%.

2.5.3. Biosorption isotherms

Complete biosorption isotherms were obtained by soaking 0.2 g of wet bacteria in a series of flasks containing 100 mL of Th(IV) ion solution with different concentrations. The initial pH of each solution was adjusted at 4.5 ± 0.1. The flasks were conditioned at 300 rpm while keeping the temperature at 25°C for 30 min. Later on, the residual concentration of Th(IV) ion was determined following the above method. Each data point was taken as the average of three measurements with standard deviation of 2 ± 0.5%.

2.6. Transmission electron microscopic determination

Control and accumulated living and dead bacterial biomass samples were prepared for the electron microscopic determination according to the method recommended by Philipp [35]. The electron microscopic determination in both instances was done to illustrate and study the sites of metal ion biosorption. Moreover, for explaining the mechanism of metal ion biosorption, both living and dead *C. cellulans* were taken for electron microscopic examination.

3. Results and discussion

3.1. Radioactivity of the wastewater sample

The concentrations of the radioactive isotope in wastewater sample from which *C. cellulans* was isolated are shown in Fig. 1 and were found to be: $^{235}U_{92}$ ≈ 4.9, $^{238}U_{92}$ ≈ 2.6, and $^{137}Cs_{55}$ ≈ 2.9 Bq/L; these values show a detectable radioactivity for the used water sample.

3.2. Characterization of C. cellulans

The electron micrograph of the (control) living *C. cellulans* after 2 h from the beginning of the experiment showed normal appearance (Fig. 2a), while the electron micrograph of control dead *C. cellulans* showed a slight deterioration of cell walls (Fig. 3a). As shown in Fig. 2b the electron micrographs of living *C. cellulans* in equilibrium with the studied metal ion solution showed a homogeneous strongly electron-

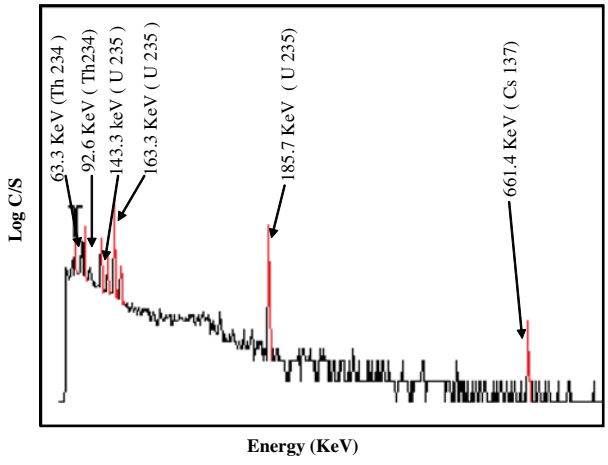

Fig. 1. γ-Spectra of the wastewater sample.

dense area on the outer section of cell walls in most of the bacterial cells. The appearance of the dense area band indicated that the bacterial cells had retained most of the sorbed studied ions.

The electron micrograph of dead *C. cellulans* cells in equilibrium with Th(IV) ion solution showed heterogeneous strongly electron dense area around the cell wall (Fig. 3b), which indicates that the cells had accumulated Th(IV) on their walls. Electron micrographs of living and dead *C. cellulans* accumulated by Th(IV) showed that the cells were dark in color. This is due to the biosorption of Th(IV) which is an α emitter, inside the cells.

Zeta potential measurements, presented in Fig. 4, indicate that, under acidic conditions, dead *C. cellulans* has positive surface charge up to approximately pH of 6.1 (the point of zero charge, PZC). At higher pH values, the surface charge reverses to negative, which is due to the deprotonation of active site in the cell wall structure of dead *C. cellulans*.

3.3. Effect of biosorbent dose

Adsorption of Th(VI) as a function of adsorbent dose is shown in Fig. 5. Obviously, as the biosorbent dose increases, the removal of Th(VI) increases. The maximum uptake was obtained at an adsorbent dose of 0.1 g, while the minimum was at 1.0 g. The observed decrease in uptake per gram of *C. cellulans* as the biosorbent dose increases may be attributed to the increased competition between the available sites for the uptake of Th(VI) compared with the small adsorbent dose. On the other hand, the uptake of Th (VI) by the dead *C. cellulans* is higher than living *C. cellulans*. This may be due to the increase of the available active sits for interaction with metal ion after the deterioration of cell walls.

3.4. Effect of time

3.4.1. Living C. cellulans

Fig. 6 shows the change in the uptake of Th(IV) by living *C. cellulans* as a function of time at initial concentrations of 111.02, 222.04, and 444.08 mg/L, at pH 4.50 ± 0.20, and temperature $25 \pm 3^{\circ}$C. It could be seen that about 90% of the total uptake of Th(IV) could be achieved within 4 min and for the initial concentrations of 111.02, 222.04, and 444.08 mg/L. While 90% of the total uptake of Th(IV) was removed after 90 min from the concentration of 888.16 mg/g. As shown in Fig. 6 1 g of living *C. cellulans* could accumulate 40.13, 80.66, 140.56, and 186.75 mg thorium from 111.02, 222.04, 444.08 and 888.16 mg/L Th(IV) solutions, respectively.

Microbial heavy metal biosorption often comprised of two phased. An initial rapid phase involving physical adsorption at the cell surface and a subsequent slower phase involving active metabolism-dependents transport of metal into bacterial cells (i.e. intracellular sequestration within specific organelles [17] or passive diffusion into the cell).

3.4.2. Dead C. cellulans

Fig. 7 shows the change in the uptake of Th(IV) by dead *C. cellulans* as a function of time at initial concentrations of 111.02, 222.04, and 444.08 mg/L, at pH 4.5 ± 0.20, and temperature $25 \pm 3^{\circ}$C. It could be seen that about 90% of the total uptake of Th(IV) could be achieved within 2 min and for the initial concentrations of 111.02 and 222.04 mg/L. While 90% of the total uptake of Th(IV) was removed after 10 min from the concentrations of 444.08 and 888.16 mg/g. As shown in Fig. 7 one gram of dead *C. cellulans* could accumulated 52.70, 105.54, 172.78, and 188.88 mg tho-

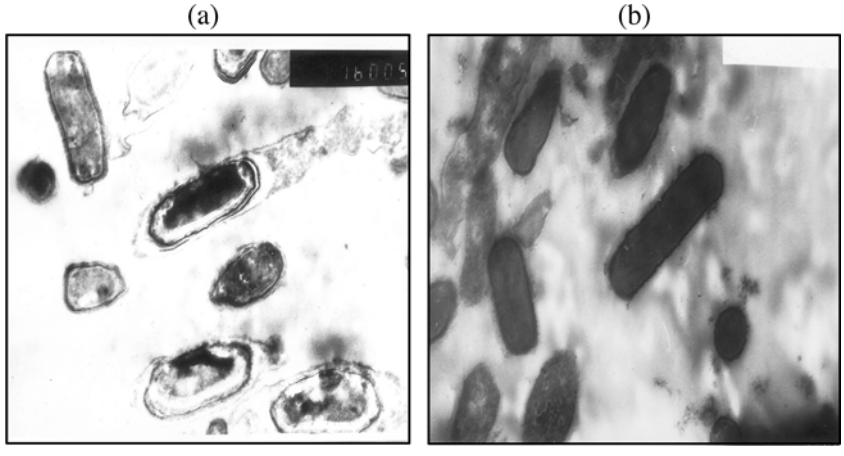

Fig. 2. Electron micrograph of living *C. cellulans*; (a) control bacterial cells, (b) accumulated bacterial cells.

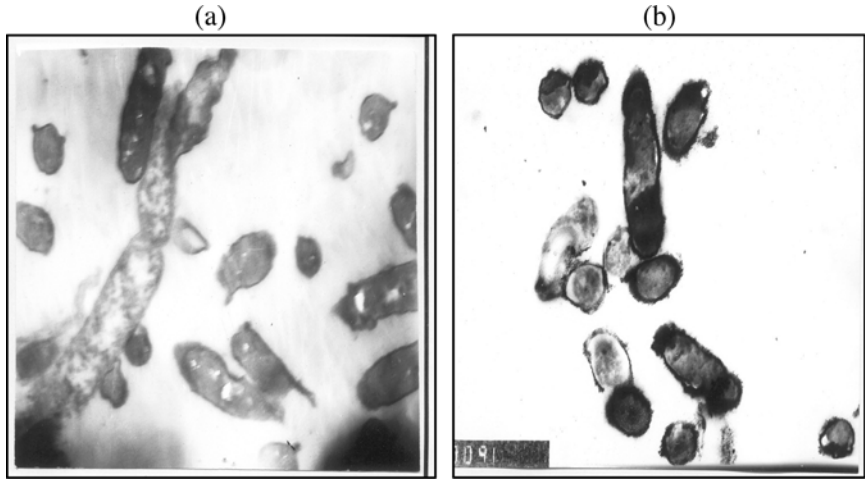

Fig. 3. Electron micrograph of dead *C. cellulans*; (a) control bacterial cells, (b) accumulated bacterial cells.

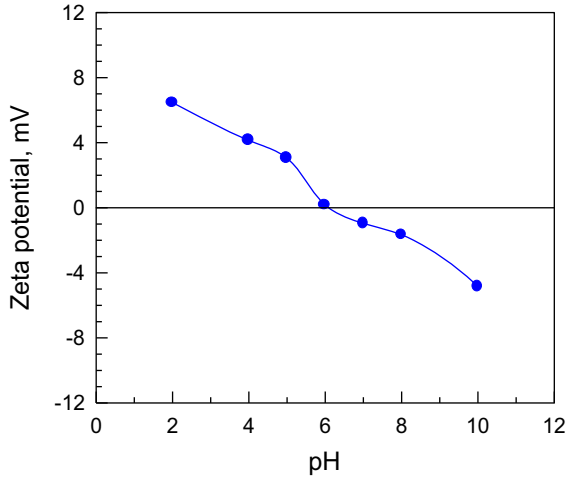

Fig. 4. Zeta potential measurements of dead *C. cellulans*, ionic strength 1×10^{-2} M NaCl.

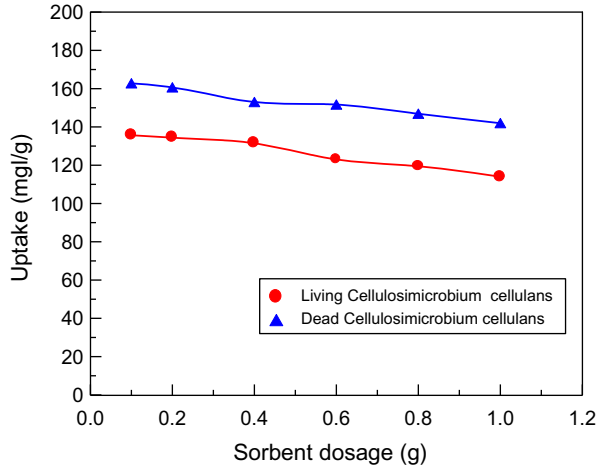

Fig. 5. Uptake of Th(IV) as a function of adsorbent dose at initial concentration of 444.08 mg/L.

rium from 111.02, 222.04, 444.08, and 888.16 mg/L Th (IV) solutions, respectively.

The bacterial cell wall contains polysaccharides, proteins and lipid and could be considered as active sits with amino, carboxyl, phosphate, and sulfate groups [36]. The biosorption of heavy metals by bacterial cells could be explained by interaction between metal cations and functional groups of cell wall constituents [37,38].

The capacity of thorium ion uptake by the dead cells was greater than that of living cells, which is clear in the second phase of the uptake process. Moreover, the application of dead cells offers several advantages over living cells due to the sensitivity of living cells in adverse condition such as toxicity effect of metal concentration, pH, and temperature, and

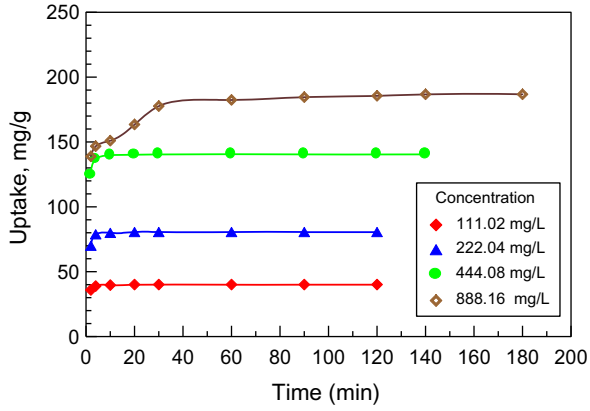

Fig. 6. The accumulated amount of Th(IV) in mg/g of living *C. cellulans* at different contact time, at pH 4.5 ± 0.2 and temperature 25 ± 3°C.

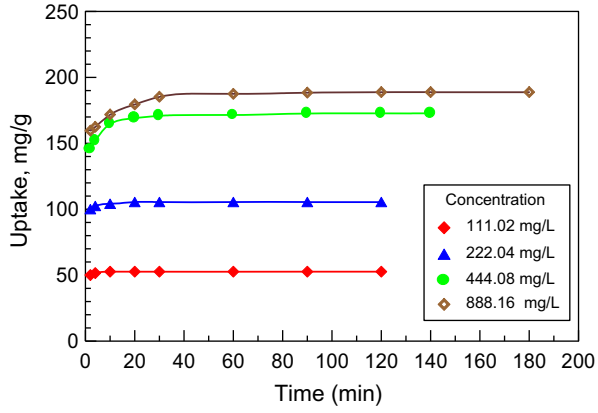

Fig. 7. The accumulated amount of Th(IV) in mg/g of dead *C. cellulans* at different contact time, at pH 4.5 ± 0.2 and temperature 25 ± 3 °C.

continuity in nutrient supply. It can be regenerated and reused for a number of cycles.

3.5. Kinetic studies

The data in Figs. 6 and 7 were treated according to pseudo-first- and pseudo-second-order kinetic models.

(i) Pseudo-first-order model [39]:

$$\log(q_e - q_t) = \log q_e - \left(\frac{k_1}{2.303}\right)t \quad (3)$$

where k_1 is the pseudo-first-order rate constant (min^{-1}) of adsorption and q_e and q_t (mg/g) are the amounts of dye adsorbed at equilibrium and time t, respectively.

(ii) Pseudo-second-order model [40]:

$$t/q_t = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right)t \quad (4)$$

where k_2 is the pseudo-second-order rate constant of adsorption (g/mg min). The kinetic parameters in both two models are determined from the linear plots of log (q_e–q_t) vs. t for pseudo-first order or (t/q_t) vs. t for pseudo-second order. The validity of each model is checked by the fitness of the straight line (R^2) as well as the experimental and calculated values of q_e. Accordingly, and as shown in Table 1, pseudo-second-order model (Eq. (6)) is more valid for adsorption process than the pseudo-first-order one (Eq. (5)) (the values of q_e of pseudo-second order-model are more comparable with the experimental ones). This behavior implies the dependence of the reaction rate on the textural properties of the studied bacteria.

Table 1
Parameters of the pseudo-first order and pseudo-second order for the biosorption of Th(IV) onto *C. cellulans*

Biosorbent	Concentration (mg/L)	$q_{e,\,exp}$ (mg/g)	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
			k_1 (min^{-1})	q_e (mg/g)	R^2	k_2 (g/mg min)	q_e (mg/g)	R^2
Living cells	111.02	40.13	0.247	4.910	0.8,697	0.219	40.182	0.9,999
	222.04	80.66	0.298	11.600	0.9,608	0.104	80.771	0.9,999
	444.08	140.56	0.192	10.495	0.9,355	0.069	140.699	0.9,999
	888.16	186.75	0.032	40.823	0.9,610	0.002	188.662	0.9,998
Dead cells	111.02	52.7	–	–	–	0.405	52.728	0.9,999
	222.04	105.54	0.167	6.776	0.9,668	0.112	105.641	0.9,999
	444.08	172.78	0.056	18.561	0.9,307	0.011	173.438	0.9,999
	888.16	188.88	0.047	25.854	0.9,671	0.006	189.830	0.9,999

Most biosorption reactions take place through a multistep mechanism comprising (i) external film diffusion, (ii) intraparticle diffusion (active metabolism or passive diffusion), and (iii) interaction between adsorbate and active site. Since the first step is excluded by shaking the solution, the rate-determining step is one of the other two steps.

3.6. Biosorption isotherm

Fig. 8 shows the isotherms of biosorption of Th(VI) on living and dead *C. cellulans*, respectively. The biosorption curves show maximum uptake values of 151.94 and 220.56 mg/g for living and dead *C. cellulans*, respectively. The adsorption data were plotted according to Langmuir equation [41].

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{K_L Q_{max}} \quad (5)$$

where C_e is the equilibrium concentration of metal ions in solution (mg/L), q_e is the adsorbed value of metal ions at equilibrium concentration (mmol/g), Q_{max} is the maximum adsorption capacity (mmol/g), and K_L is the Langmuir binding constant, which is related to the energy of adsorption (L/mg). Plotting C_e/q_e against C_e gives straight lines, indicating that the adsorption complies with the Langmuir isotherm as. Each straight line has slope and intercept equal to $1/Q_{max}$ and $1/K_L Q_{max}$, respectively. The values of K_L and Q_{max} for the biosorption of Th(VI) are reported in Table 2. It is seen that the value of Q_{max} (obtained from Langmuir plots) is mainly consistent with that experimentally obtained, indicating that the biosorption process is mainly monolayer. The observed R^2 values indicate that the adsorption of Th (VI) correlates well with the Langmuir model.

Alternatively, the experimental data in Fig. 8 may be analyzed with the Freundlich isotherm model, which in its linearized form is [42]:

$$\log q_e = 1/n \log C_e + \log K_f \quad (6)$$

where K_f and n are Freundlich constants, K_f and $1/n$ indicate the biosorption capacity and biosorption intensity, respectively. Freundlich constants were

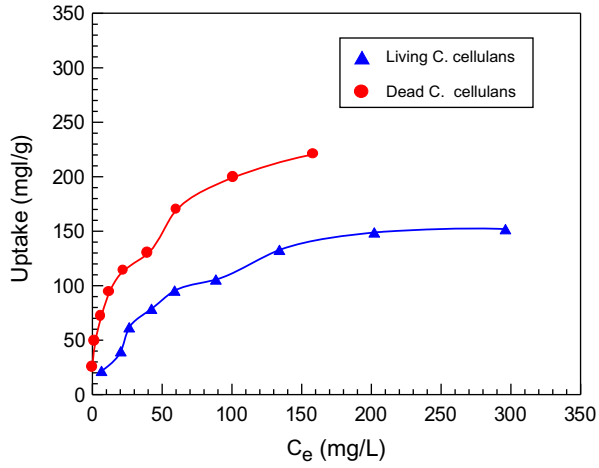

Fig. 8. Isotherms for the biosorption of Th(IV) in mg/g of living and dead *C. cellulans* at pH 4.5 ± 0.2 and temperature 25 ± 3°C.

obtained from the plot of $\log q_e$ vs. $\log C_e$ for the experimental data in Fig. 8 and are reported in Table 3. It illustrates that Th(VI) ion biosorption onto *C. cellulans* obeys the Freundlich isotherm model reasonably well. The magnitude of n gives an indication of the favorability of adsorption. Values of $n > 1$ represent favorable adsorption condition. The higher the n value, the stronger the biosorption intensity [42]. Table 3 shows that the values of the Freundlich exponent n are 1.902 and 3.653 for living and dead *C. cellulans*, respectively, indicating that the biosorption process is favorable. The n value of dead *C. cellulans* is higher than that of living *C.* cellulans, indicating stronger biosorption intensity in dead *C. cellulans*. It is observed that the Langmuir isotherm model is more suitable for the experimental data than Freundlich isotherm when comparing the correlation coefficient values (R^2) of Langmuir and Freundlich isotherms. These data confirm that the biosorption of Th(VI) on *C. cellulans* occurs as monolayer coverage process.

3.7. Mechanism of biosorption process

Biosorption of metal ions onto micro-organisms involves a combination of the following metal-binding mechanisms including physical adsorption, ion

Table 2
Langmuir constants for biosorption of Th(IV) onto the studied *C. cellulans*

Living cells				Dead cells			
$Q_{max,\ exp}$ (mg/g)	$Q_{max,\ calc}$ (mg/g)	K_L (L/mg)	R^2	$Q_{max,\ exp}$ (mg/g)	$Q_{max,\ calc}$ (mg/g)	K_L (L/mg)	R^2
151.94	184.456	0.017	0.9920	220.56	227.630	0.112	0.9,883

Table 3
Freundlich constants for biosorption of Th(IV) onto the studied *C. cellulans*

Living cells			Dead cells		
K_f	n	R^2	K_f	n	R^2
9.524	1.902	0.948	50.058	3.653	0.963

Table 4
Biosorption of thorium by different biosorbents

Biosorbent	Maximum adsorption capacity (mg/g)	Reference
Sargassum filipendula	6.14	[43]
Aspergillus fumigatus	370	[44]
Aspergillus niger	22	[45]
Aspergillus terus	60	[45]
Penicillium chrysogenum	142	[45]
Rhizopus arrhizus	185	[45]
Saccharomyces cerevisiae	119	[46]
C. cellulans	220	This work

exchange, complexation, and precipitation [18,22]. Each mechanism is described as follows:

1. van der Waals forces (electrostatic interaction) may be responsible for the biosorption uptake of positive Th(VI) ions into the cell wall of *C. cellulans*.
2. Complex formation on the cell surface of *C. cellulans* after the interaction between Th(VI) ions and active sites. Th(VI) can be biosorbed or complexed (formation of coordination bonds between metals and carboxyl and amino groups of the cell wall) by carboxyl groups found in the microbial polysaccharides or other polymers.
3. Polysaccharides that existed on the cell wall of micro-organisms consist of counterions such as K (I), Na(I), Ca(II), and Mg(II). These ions can exchange with Th(VI) ions resulting in metal ions uptake.
4. Precipitation of Th(VI) ions from aqueous solution often associates with active defence system of micro-organisms. This active system is a system that produces compounds favoring the precipitation of Th(VI) [17].

Therefore, the amount of biosorbed Th(VI) onto dead and living *C. cellulans* is basically the combined effect of the above mentioned four mechanisms.

3.8. Comparison with different biosorbents

Although the data reported in Table 4 showed appreciable sorption of Th(VI) ions onto different biomasses (fungi and bacteria), it may be noticed that *C. cellulans* has high efficient sorption capacity among the biosorbents (220 mg/g). This result indicates that *C. cellulans* is a promising sorbent for the future removal of Th(VI) ions from radioactive wastewater.

4. Conclusion

The removal of Th(IV) from aqueous solutions using living and dead *C. cellulans* was studied. The obtained results showed that living and dead *C. cellulans* could sorp 151.94 and 220.56 mg/g, respectively. These data indicate that *C. cellulans* is a very efficient sorbent for Th(IV) removal. Electron microscopic examinations of living and dead *C. cellulans* before and after biosorption of thorium ions indicate that the cells had accumulated Th(IV) on their walls. The adsorption of Th(IV) by bacterial cells could be explained by interaction between metal cations and negative charge of acidic functional groups of cell wall constituents. Kinetic studies referred to the fact that the adsorption reaction is pseudo-second order and the biosorption process proceeds according to the Langmuir isotherm.

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