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Biosorption Mechanism of Cadmium by Acid-Treated Non-Living *Rhodopseudomonas* sp. Strain SBL

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Abstract

This research aimed to evaluate the effects of initial cadmium concentration and contact time on the biosorption capacity of cadmium by acid-treated non-living *Rhodopseudomonas* sp. strain SBL, to analyze the mechanism of the biosorption by kinetic models, and to characterize the morphological and functional group changes of *Rhodopseudomonas* sp. strain SBL in the biosorption process. The biosorption capacity of cadmium was quantitatively evaluated using an Atomic Absorption Spectrophotometer (AAS). The biosorption data were fitted to pseudo-first-order and pseudo-second-order models to assess the biosorption process's mechanism. Changes in the morphological and functional groups of Rhodopseudomonas sp. strain SBL after biosorption were examined using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR), respectively. The effect of initial cadmium concentration indicated that the percentage of biosorption declined as the initial cadmium concentration increased, with an initial concentration of 0.5 mg/L exhibiting the highest biosorption capacity of 77.5%, and a cadmium uptake of 17.95 mg Cd+/mg biomass. The effect of contact time showed that the percentage of biosorption increased with contact time, reaching equilibrium after 30 minutes. In the biosorption kinetic study, the obtained data fit the pseudo-second-order model, suggesting that the biosorption process involves chemical interactions with the biosorbent. The observation under SEM showed morphological changes of *Rhodopseudomonas* sp. strain SBL from smooth to rough surfaces, with the formation of pores following the biosorption process. The FTIR analysis showed hydroxyl, carbonyl, and carboxyl groups as the main functional groups involved in the biosorption process. The findings of this study suggested that the acid-treated non-living Rhodopseudomonas sp. strain SBL holds promising potential as a biosorbent for removing cadmium through biosorption.

Keywords biosorption, adsorption mechanism, cadmium, *Rhodopseudomonas* sp.

1.0 INTRODUCTION

Increased urbanization and industrialization have resulted in increased environmental heavy metal pollution. Non-essential heavy metals, such as cadmium, are environmental pollutants that cannot be degraded naturally and induce toxicity in living organisms (Swain, 2024). The detrimental impact of cadmium poses a serious issue for both public health and the environment.

Several studies have reported that purple non-sulphur bacteria (PNSB) are resistant to heavy metals and can remove them (Qian et al, 2024; Armadi et al, 2024). According to Dhar et al. (2023), PNSB strains such as *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* have demonstrated practical bioremediation of several toxic and carcinogenic heavy metals, including cadmium. Studies on heavy metal removal by PNSB have been abundant, ranging from mercury, chromium, cobalt, nickel, and cadmium. The biosorption capacities for these heavy metals by PNSB range from 80% to 90 % removal in aqueous solution (Gao et al., 2019; Mukkata et al., 2019; Jia et al., 2022; Qian et al., 2024; Armadi et al., 2024). The significant advantages of using PNSB as biosorbents include converting to different growth modes, overcoming organic

nutrient limitations, and adapting to diverse growth conditions (Dhar et al., 2023). Therefore, the potential of locally isolated PNSB species in the biosorption of cadmium needs to be investigated.

While living cells are typically used as biosorbents, the use of dead biomass (non-living strain) is preferred because it does not require a nutrient supply, operates effectively over a wide pH range, and has a greater biosorption capacity than living biomass. (Pande et al., 2022). The biosorption efficiency of biomass is also often enhanced through an acid pretreatment step, as it could increase the uptake of metal ions through the incorporation of acidic functional groups and increase surface area (Yan et al, 2024) Biosorption generally occurs through several interactions, including physical adsorption, ion exchange, complexation, and precipitation (Milano et al., 2024). The biosorption process in non-living bacterial cells occurs via exopolysaccharides (EPS) or cell walls (Chauhan et al., 2020). This study explored the ability of acid-treated non-living *Rhodopseudomonas* sp. strain SBL as a potential biosorbent for cadmium, offering a more sustainable approach to bioremediation.

2.0 EXPERIMENTAL

2.1 Preparation of Acid-Treated Non-Living Biomass

The biomass cultivated for 7 days at 30°C under facultative light conditions in purple non-sulphur bacteria enrichment medium (PNSBEM) was collected via centrifugation at 9000 rpm and 4°C for 10 minutes. The cell pellets were then washed twice with sterile deionized water. Next, 10 mL of 0.1 M HCl was added to the pellets and incubated at 150 rpm for 10 minutes. The resulting cell suspension underwent centrifugation again at 9000 rpm and 4°C for 10 minutes. The pellets were washed with sterile deionized water and re-centrifuged three times. Finally, the pellets were dried in an oven at 70°C for 15 hours and ground into powder with a mortar and pestle (Asadi Haris et al 2018).

2.2 Preparation of Cadmium Stock Solution

A cadmium stock solution of 1000 ppm was prepared by dissolving 2.7442 g of cadmium nitrate tetrahydrate, $Cd(NO_3)_2.4H_2O$, in 1000 mL of deionized water containing 1% (v/v) of nitric acid, HNO_3.

2.3 Effect of Different Parameters on the Biosorption Process

2.3.1 Biosorption Experiment

Approximately 0.5 mg of treated biomass was added to 30 mL of a 1.0 mg/L cadmium solution at pH 7 and incubated at 30°C for 30 minutes under facultative light conditions. Following this, the cell suspension was centrifuged at 8000 rpm for 15 minutes to separate the supernatant (Li et al., 2018). The final cadmium concentration in the supernatant was analyzed using a Perkin Elmer AA-6300 atomic absorption spectrophotometer (AAS). These experiments were conducted in triplicate and repeated with initial cadmium concentrations of 0.5, 0.6, 0.8, and 1.0 mg/L as well as contact times of 15, 30, 45, and 60 minutes.

2.3.2 Quantitative Analysis

The concentration values were used to calculate the percentage biosorption of cadmium using the following formula:

$$\text{Biosorption (\%)} = [(C_i - C_f)/C_i] \times 100 \quad (1)$$

where C_i is the initial concentration of Cd in the solution, and C_f is the final concentration of metal ions in the solution (Asadi Haris et al., 2018).

$$\text{Quantitative metal sorption (mg } Cd^+ \text{ / g biomass)} = [\, V\,(C_i - C_f)/\, m] \quad (2)$$

Where V is the total volume (mL) of the solution, C_i (mg/L) is the initial concentration of Cd in the solution, C_f (mg/L) is the equilibrium concentration of metal ions in the solution, and m is the mass of biomass (g) (Mukatta et al., 2019).

2.4 Kinetic Models

The final concentration value from section 2.3 was then fitted with pseudo-first-order and pseudo-second-order models.

$$q_t = q_e\,(1 - e^{-k1t}) \quad (3)$$

$$t/q_t = [1/k_2q_2e] + t/q_e \quad (4)$$

Where q_t and q_e are the amount of metal adsorbed at any time (min) and equilibrium (mg/g), respectively, and K_1 (min^{-1}) and K_2 (g/mg/min) are pseudo-first order and pseudo-second-order rate constants, respectively (Mukatta et al., 2019).

2.4.1 Nonlinear Kinetic Modeling

The kinetic data were fitted using nonlinear regression to two common adsorption kinetic models: the pseudo-first-order model and the pseudo-second-order model.

The nonlinear form of the pseudo-first-order model is:

$$q_t = q_e(1 - e^{-k_1 t}) \qquad (4)$$

Where q_e is the equilibrium adsorption capacity (mg/L), k_1 and the pseudo-first-order rate constant (min^1).

The nonlinear form of the pseudo-second-order model is:

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t}$$

Where k_2 is the pseudo-second-order rate constant (L/mg/min).

The kinetic parameters (k_1, k_2, q_e) were obtained by fitting the experimental data to the models using the Levenberg–Marquardt algorithm implemented in Python's scipy.optimize.curve_fit function. The goodness-of-fit for each model was evaluated based on the coefficient of determination (R^2). Models with higher R^2values and physically meaningful parameters were considered more appropriate to describe the biosorption process.

2.5 Characterization of *Rhodopseudomonas* sp. strain SBL

2.5.1 Scanning Electron Microscopy (SEM)

*T*he morphology of *Rhodopseudomonas* sp. strain SBL was observed before and after the biosorption experiment. The biomass prepared without contact with the cadmium solution serves as a sample before the biosorption process. In contrast, the biomass subjected to a biosorption experiment at an initial cadmium concentration of 1.0 mg/L with a contact time of 30 minutes represents a sample after the biosorption process. The cell pellets were collected by centrifugation at 8000 rpm for 15 minutes and underwent sample pre-treatment. The pellets were combined with 50 mL of phosphate-buffered saline (PBS) and centrifuged at 4000 rpm for 15 minutes. The supernatant was discarded, and 1 mL of 2.5% glutaraldehyde was added, then kept at 4°C for 20 hours. Subsequently, 50 mL of PBS was added to the pellet and centrifuged at 4000 rpm for 15 minutes. The supernatant was removed, and 2 mL of a series of ethanol concentrations of 50, 60, 70, 80, 90, and 100% was discarded every 10 minutes. Finally, the pellets were freeze-dried before analysis using scanning electron microscopy (SEM), JSM-5510LV, JEOL (Ramrakhiani et al., 2016).

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

The surface of Rhodopseudomonas sp. strain SBL was examined before and after the biosorption experiment. The biomass prepared without contact with the cadmium solution serves as a sample before the biosorption process. In contrast, the biomass subjected to a biosorption experiment at an initial cadmium concentration of 1 mg/L and a contact time of 30 minutes serves as a sample after the biosorption process. The cell pellets were collected by centrifugation at 8000 rpm for 15 minutes and dried in an oven at 70°C for 15 hours. The dried pellets were treated with KBR and scanned in the 650 to 4000 cm^{-1} range using Perkin Elmer Fourier transform infrared spectroscopy (FTIR) (Asadi Haris et al., 2018).

3.0 RESULTS AND DISCUSSION

3.1 Effect of Different Parameters on the Biosorption Process

3.1.1 Effect of Initial Cadmium Concentration

Figure 1 illustrates that the percentage of biosorption decreased as the initial cadmium concentration increased, with an optimal initial concentration of 0.5 mg/L achieving 77.5% biosorption capacity. The metal sorption at this concentration is the

lowest, at 17.95 mg Cd+/g biomass. The higher biosorption capacity at lower initial cadmium concentrations is due to the availability of more active sites (Hariharan et al., 2020). However, as the initial cadmium concentration increases, the biosorption efficiency declines because of the rising cadmium ions. In contrast, the number of binding sites on the biosorbent remains constant, leading to the saturation of surface-active sites (Asadi Haris et al., 2018). Ultimately, this results in the diffusion of metal ions into the interior part of the biomass, causing a slower adsorption rate.

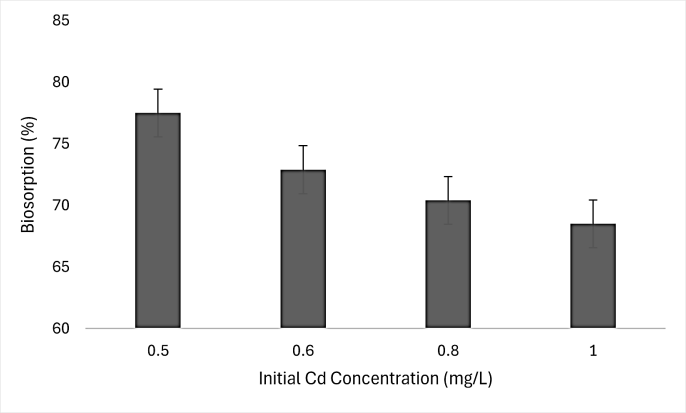

Figure 1 Effect of initial cadmium concentration on biosorption capacity

The quantitative metal sorption of this acid-treated biomass indicated that as the initial cadmium concentration increases, the value of metal sorption rises to 24.2 mg Cd^{+}/g biomass at a Cd concentration of 0.6 mg/L, 26.5 mg Cd^{+}/g biomass at a Cd concentration of 0.8 mg/L, and reaches its highest at a Cd concentration of 1 mg/L with the metal sorption of 41.8 mg Cd^{+}/g biomass. This occurs due to the accumulation of Cd^{+} on the surface of the biomass as the concentration of Cd^{+} increases. The considerably higher concentration gradient pushes more Cd^{2+} onto the biomass, even after forming the initial monolayer, resulting in a significantly higher sorption capacity (Van et al., 2019).

3.1.2 Effect of Contact Time

According to Figure 2, the percentage biosorption increased with contact time and reached equilibrium after 30 minutes, with a contact time of 30 minutes giving the highest biosorption capacity of 68.5%, 1.4 mg Cd^{+}/ g biomass. The rapid increase at minute 30 can also be seen in previous studies by Li et al. (2023) on cadmium biosorption using *Cupriavidus necator* GX_5.

The increase in biosorption capacity from minute 0 to 30 may be attributed to the abundance of binding sites and a greater driving force due to a steep concentration gradient between metal ions in the bulk solution and those on the biosorbent surface. Conversely, the decline in biosorption efficiency after 30 minutes is a result of the saturation of binding sites by the end of the experiment (Mandal et al., 2020). Additionally, the release of metal ions from the biosorbent surface into the solution and the diffusion of metal ions into the inner part of the biomass may contribute to the reduced biosorption capacity. This results in a decreased number of metal ions bound to the biosorbent, leading to lower biosorption efficiency.

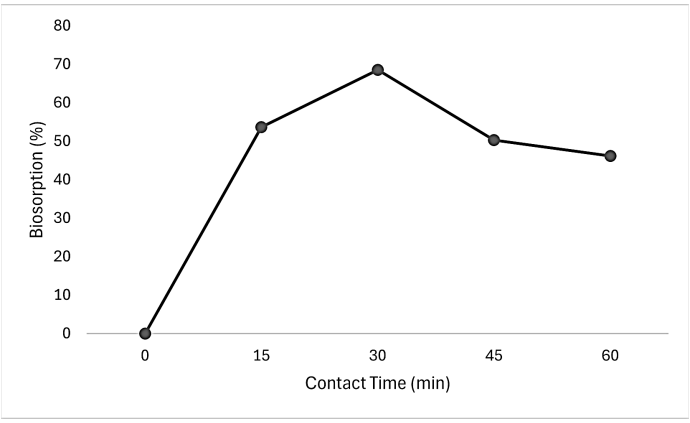

Figure 2 Effect of contact time on biosorption capacity (initial cadmium concentration: 1 mg/L)

The data is compared to non-treated biomass and shows no significant difference in biosorption capacity and metal sorption of Cd. At 30 minutes, the biosorption capacity was 65%, and the Cd sorption was at 11.11 mg Cd^{+}/ g biomass. Acid treatment can increase the biosorption capacity by enhancing the binding affinity of the biomass for Cd ions. This result contradicts the findings by Hashem et al. (2021), which show that Cd biosorption is more effective with acid-treated biomass. Moreover, some studies show that acid treatment can lead to a lower adsorption capacity than alkaline treatment (Yan et al., 2024). This indicates that the biomass of *Rhodospeudomas* strain SBL does not need to be acid-treated or could be improved by alkaline pretreatment for application in the bioremediation of Cd from aqueous solution.

3.2 Biosorption Kinetic Models

According to Atlas et al. (2024), the appropriate model for this study was selected based on the correlation factor. A higher correlation factor indicates a better fit of the model for analysing the adsorption process. The data obtained could not fit the biosorption linear model for the pseudo-first-order and pseudo-second-order models. Therefore, the kinetics of biosorption were analysed using both pseudo-first-order and pseudo-second-order nonlinear models.near models.

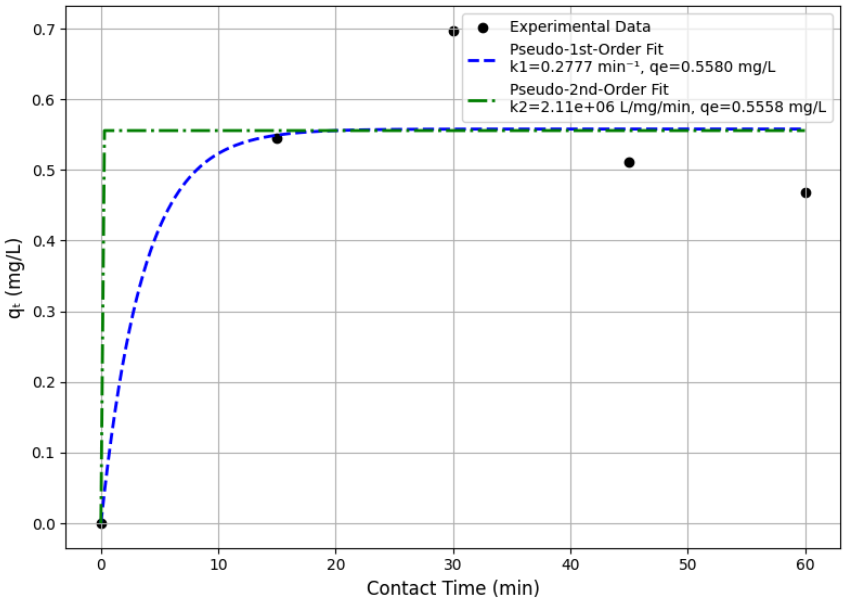

Figure 4 Non-linear kinetic model of pseudo-first-order (PFO) and pseudo-second-order (PSO) cadmium biosorption generated using Python via Google Colab with code provided by OpenAI's ChatGPT (2025).

The nonlinear pseudo-first-order model yielded a rate constant (k_1) of 0.2777 min^{-1} and an equilibrium uptake capacity (q_e) of 0.5580 mg/L, with a correlation coefficient (R^2) of 0.8937. Similarly, the nonlinear pseudo-second-order model produced a rate constant (k_2) of 1,427,691.72 L/mg/min and a q_e value of 0.5558 mg/L, with an R^2 of 0.8933. Although both models fit the experimental data well, the pseudo-second-order model produced an unrealistically high rate constant, indicating a less reliable interpretation. Therefore, the pseudo-first-order kinetic model more accurately describes the biosorption process of cadmium by acid-treated non-living Rhodopseudomonas sp. strain SBL. The high R^2 value indicates that the biosorption follows a relatively fast adsorption mechanism initially, reaching near-equilibrium within the studied timeframe. By fitting into the pseudo-first model, it indicates that the biosorption process of the biomass occurs through chemisorption, which involves valency forces by sharing electrons between transition metal cations and the surface of the adsorbent. (Ngah et al., 2004). This demonstrated that chemical adsorption was the rate-limiting step in biosorption (Ma et al., 2015). This result is consistent with Mukkata et al. (2019), who reported that biosorption by microbes is often associated with pseudo-second-order models.

3.3 Characterization of *Rhodopseudomonas* sp. strain SBL

3.3.1 Scanning Electron Microscopy (SEM)

Figure 5 shows the morphology of acid-treated non-living *Rhodopseudomonas* sp. strain SBL before the biosorption experiment. The biomass surface exhibited well-defined rod shapes with smooth surfaces.

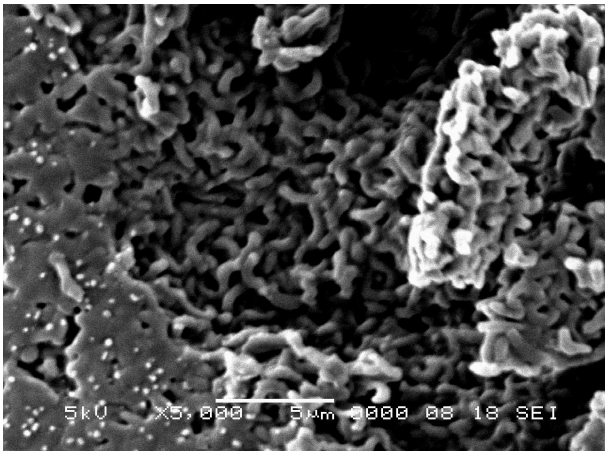

Figure 5 SEM micrograph of *Rhodopseudomonas* sp. strain SBL before cadmium biosorption at 5000x magnification

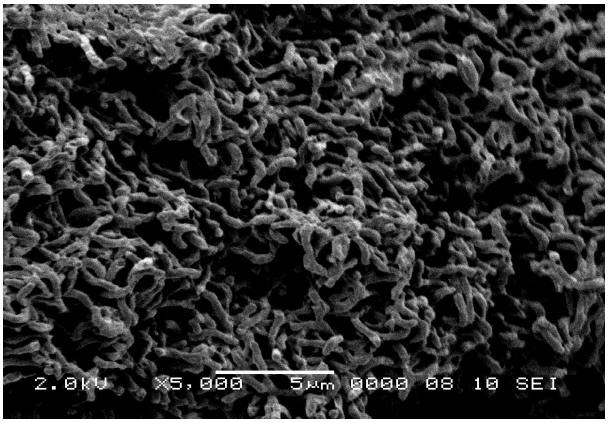

Figure 6 SEM micrograph of *Rhodopseudomonas* sp. strain SBL after cadmium biosorption at 5000x magnification.

After biosorption, the surface of *Rhodopseudomonas* sp. strain SBL changed from smooth to rough, showing deformed or shrunk rod shapes and the formation of pores, as demonstrated in Figure 6. This is due to the precipitation of cadmium ions on the cell surface and the cross-linking of functional groups with metal ions, leading to structural changes, creating a more rigid structure (Shen et al., 2021).

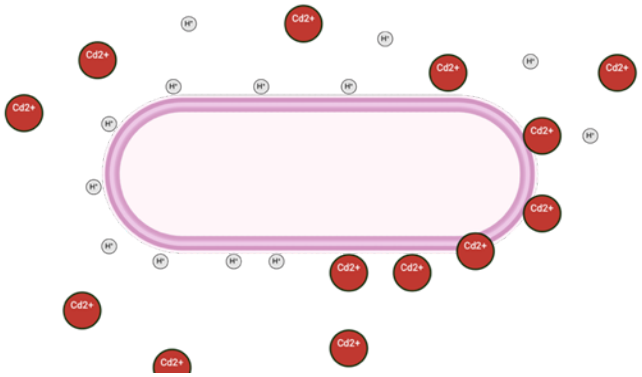

Figure 7 Mechanism of cadmium ion towards acid-treated biosorbent (*Rhodospeudomonas* strain SBL)

Moreover, changes in cell morphology could also be viewed as a protective mechanism against a stressful environment, as previously reported (Qian et al., 2024; Jia et al., 2022). Alterations in the surface morphology suggest that Rhodopseudomonas sp. strain SBL adsorbed cadmium ions during biosorption. This observation aligns with previous studies in which the surface of the biosorbent shifted from smooth to rough upon contact with cadmium ions (Shen et al., 2021; Satya et al., 2020).

3.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Based on Figure 8, the FTIR spectra showed shifts in peak position and intensity before and after the biosorption process. The absorption peak changes indicate a metal binding process on the surface of acid-treated non-living *Rhodopseudomonas* sp. strain SBL.

This occurs due to complexation between functional groups and cadmium ions, causing chemical structure modifications (Petrović et al., 2016). This suggests the involvement of chemisorption and ion-exchange mechanisms during cadmium adsorption on the biosorbent. The results indicated the involvement of functional groups, mainly hydroxyl (shifted from 3269.34 cm^{-1} to 3286.22 cm^{-1}), carbonyl (1628.05 cm^{-1} to 1628.85 cm^{-1}), and carboxyl (1231.04 cm^{-1} to 1230.99 cm^{-1}) groups, in the biosorption of cadmium (Roșca et al., 2023).

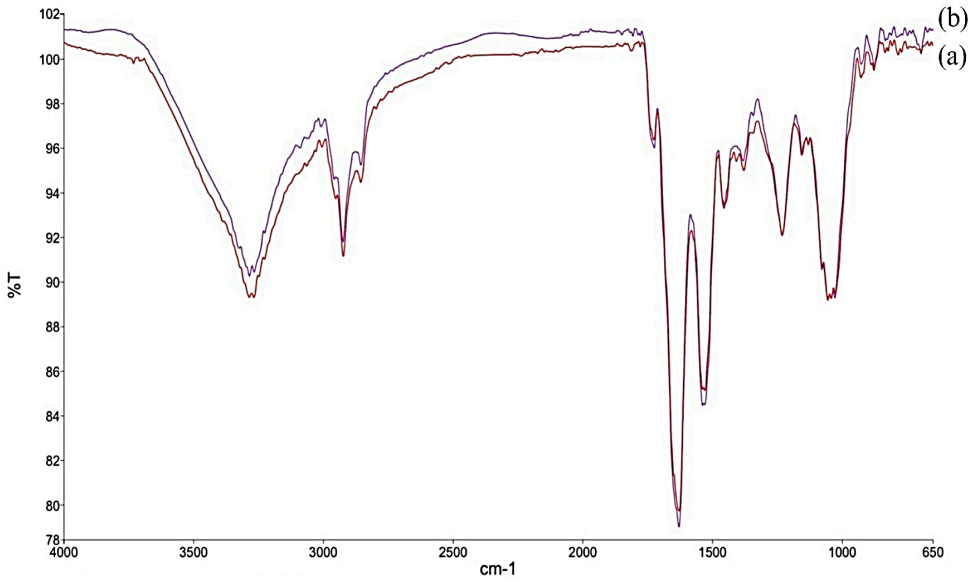

Figure 8 FTIR spectra of *Rhodopseudomonas* sp. strain SBL (a) before and (b) after cadmium biosorption

4.0 CONCLUSION

The initial cadmium concentration and contact time significantly affect cadmium biosorption capacity, with an initial concentration of 0.5 mg/L and a contact time of 30 minutes yielding the highest biosorption capacities of 77.5% and 68.5%, respectively. Kinetic studies revealed that the biosorption process fitted the pseudo-second-order model, suggesting that it occurs through chemisorption. The SEM and FTIR analyses demonstrated morphological changes in Rhodopseudomonas sp. strain SBL and the involvement of functional groups in the biosorption process, indicating modifications to the biosorbent surface following interaction with cadmium ions. Overall, the acid-treated non-living Rhodopseudomonas sp. strain SBL exhibited promising potential as a biosorbent for cadmium removal through biosorption.

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