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RESEARCH ARTICLE

Cadmium Biosorption by Non-Living Biomass of Locally Isolated *Rhodopseudomonas* sp. Strain SBL

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Abstract *Rhodopseudomonas* sp. strain SBL is a purple non-sulfur bacterium (PNSB) originating from the Proteobacteria phylum and was locally isolated from the Kim Kim River in Pasir Gudang, Johor, Malaysia. PNSBs are widely used in the bioremediation of heavy metal removal through biosorption due to their membrane surface characteristics. Biosorption is a sustainable biotechnological method for minimizing waste using natural or modified forms. The non-living biomass of *Rhodopseudomonas* sp. strain SBL has been shown to remove cadmium from aqueous solutions via biosorption effectively. In this study, the biosorption capacity of cadmium by *Rhodopseudomonas* sp. strain SBL was investigated using the one factor at a time (OFAT) method, examining various environmental factors such as biomass dosage, pH of the cadmium solution, incubation temperature, and contact time with cadmium. It was quantitatively analyzed using an atomic absorption spectrophotometer (AAS). This was followed by kinetics and morphological characterization of the cadmium biosorption by *Rhodopseudomonas* sp. strain SBL. The surface morphological changes and the functional groups involved were analyzed before and after biosorption using Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-Ray (EDX), and Fourier Transform Infrared Spectroscopy (FTIR). *Rhodopseudomonas* sp. strain SBL removed the largest amount of cadmium with 0.5 mg/mL of non-living *Rhodopseudomonas* sp. strain SBL biomass at a pH of 5 in the cadmium solution, an incubation temperature of 30°C, and a contact time of 30 minutes. The biosorption kinetics of cadmium follow a pseudo-second order model. Observations via FESEM showed morphological changes on *Rhodopseudomonas* sp. strain SBL's cell surface, and FTIR analysis indicated that hydroxyl (O-H), alkenes (C=C), and alkyl (C-F) groups were the main functional groups involved in the biosorption by *Rhodopseudomonas* sp. strain SBL. Our results highlight the potential of using the non-living biomass of the purple non-sulfur bacterium, *Rhodopseudomonas* sp. strain SBL, as a biosorbent for cadmium in solution.

Keywords: *Rhodopseudomonas* sp., purple non-sulfur bacteria, biosorption, cadmium.

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Introduction

Cadmium is a toxic heavy metal found in various environmental matrices, such as water bodies, soil, and air. Its presence is mainly due to its widespread usage in various industry sectors, such as mining, tannery, petrochemicals [15], electroplating, alloys, batteries, pigments, and plastic [3]. Additionally, human activities such as the open combustion of fossil fuel [14] and the use of phosphate fertilizers [1] have increased cadmium pollution.

According to Genchi *et al*. [14], cadmium has a long half-life of approximately 25 to 30 years and can accumulate in plants and animals, posing a risk to human health. Due to its ability to replace calcium in bones, exposure to cadmium may result in kidney damage, renal dysfunction, hepatotoxicity, genotoxicity, osteoporosis, osteomalacia, and cardiovascular diseases [2]. Furthermore, the Environmental Protection Agency (EPA) has classified cadmium as a Group B1 human carcinogen because of its role in inducing cancer by acting as an endocrine disruptor and promoting hormone-dependent tumors in the lungs and urinary organs [15].

Bioremediation is a notable method for eliminating cadmium from polluted environments, involving the biological process of reducing the severity of pollutants through biosorption, biodegradation, or bio-reduction [32]. Bacteria biomass is commonly used as a biosorbent due to its effectiveness in removing heavy metals, even at low concentrations [26]. While living or non-living biomass is typically used as biosorbents, the use of non-living microbial biomass is preferred for biosorption since it does not require nutrients or growth media, which makes it more cost-effective [16], has a minimum generation of waste sludge and reduces concerns about introducing foreign microbes that could lead to contamination [34].

Purple Non-Sulfur Bacteria (PNSB) is a microbe that can be used for bioremediation. These bacteria, originating from the Proteobacteria phylum, are anoxygenic bacteria and form purple blooms in environments with low or undetectable sulfide levels [11], [8]. PNSBs can perform various metabolic pathways, including photoautotrophic, photoheterotrophic, mixotrophic, and heterotrophic, which enable them to thrive in diverse conditions [22]. PNSB uses light as an energy source, organic carbon for biomass, and carbon dioxide fixation to maintain a balanced redox environment, depending on the substrate [29]. Additionally, PNSB can effectively treat organic wastewater by using organic substrates as a source of carbon and energy [36].

Materials and Methods

Strain Origin and Isolation of Purple Non-Sulfur Bacteria

Water samples were collected from a sampling site in Kim Kim River, located at Pasir Gudang, Johor, Malaysia (1°29'49.7"N 103°54'53.6"E). Water samples from Kim Kim River were grown in Purple Non-Sulfur Bacteria Enrichment Media (PNSBEM), according to Feng *et al*. (2007) [13], (1 g/L NH_4Cl, 0.5 g/L Na_2HPO_4, 0.2 g/L $MgCl_2$, 2.0 g/L NaCl, 2.0 g/L yeast extract and 6 mL of 80% sodium lactate in 1 L of distilled water, pH 7.0, sterilized by autoclaving at 121°C.). The mixture was incubated under anaerobic-light conditions for seven days at 30°C or until the media changed to purple. The enrichment culture was streaked into PNSBEM agar plates until pure isolates had been observed. Pure colonies were subsequently cultured onto PNSBEM agar several times until single pure colonies were obtained. Subculturing of the pure isolates was done under light conditions facultatively.

Identification of Purple Non-Sulfur Bacteria

The Promega Wizard® Genomic DNA Purification Kit was employed to extract DNA from the bacteria. The 16S rRNA gene was amplified using universal 16S rDNA primers: forward (Fd1) 5′-AGA GTT TGA TCC TGG CTC AG-3′ and reverse (rP1) 5′-ACG GCT ACC TTG TTA CGA CTT-3′. Subsequently, the amplified sequences were sent to Apical Sdn Bhd for sequencing (Sanger). The complete sequence of the isolated bacteria was analyzed to identify closely related sequences using BLAST and ClustalW. A phylogenetic tree was constructed using MEGA version 6.0 software.

Factors Affecting Non-Living Biomass of *Rhodopseudomonas* sp. Strain SBL in Biosorption

Rhodopseudomonas sp. strain SBL was grown in PNSBEM media for seven days at 30°C and under light conditions facultatively. The condition was changed from anaerobe to facultative to obtain more biomass for the biosorption experiment. Then, the solution underwent centrifugation at 3,500 rpm for 15 minutes and was rinsed with 0.1% peptone water (pH 7). The resulting cell pellet was then dried in the oven for 24 hours at an incubation temperature of 80°C [25]. The dry cell pellet (biomass) was then ground into a loose powder using a clean mortar and pestle.

The dried cell biomass was weighed until 0.5 mg. Then, it was transferred into a tube, and 10 mL of 1 mg/L cadmium solution with a pH of 7 was added. The cell was then incubated at 30°C and under light conditions facultatively for 30 minutes afterward. The cell suspensions were centrifuged at 8,000 rpm for 15 minutes [20]. The supernatant of the cell suspension was analyzed as the final cadmium concentration by using Perkin Elmer AA-6300 atomic absorption spectrophotometer, and the biosorbent cadmium was calculated quantitatively using the following formulas:

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

C_i represents the initial concentration of Cd in the solution, while C_f is the equilibrium concentration of metal ions in the solution [4].

The biosorption experiments were then continued by using non-living cells on various factors such as biomass dosage (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ mL), pH of Cd solution (pH 5, 6, 7, 8 and 9), incubation

temperature (20, 25, 30, 35 and 40°C), and contact time of 15 minutes interval [31]. The factors were also carried out by a one-factor-at-a-time approach to get the most suitable conditions and the highest percentage of biosorption.

Biosorption Kinetic Models

The results obtained from the biosorption experiments were fitted into the pseudo-first and pseudo-second order models using Prism version 9.2 software.

$$q_t = q_e (1 - e^{-k1t})$$
$$t/ q_t = [1/ k_2q_2e] + t/q_e$$

Where q_e and q_t represent the amount adsorbed per unit mass (mg/g) at equilibrium and at any time. K_1 (min) and K_2 (g/ mg/ min) denote the pseudo-first and pseudo-second order adsorption rate constants, respectively [25].

Characterization of *Rhodopseudomonas* sp. Strain SBL Surface as Biosorbent

FESEM and EDX Analyses

The observations of surface morphology changes of *Rhodopseudomonas* sp. strain SBL were carried out before (sample A) and after (sample B) the biosorption experiments performed by *Rhodopseudomonas* sp. strain SBL.

The dry cell biomass was prepared as described in the previous section. Biomass sample B will undergo biosorption experiments. Then, both samples were dried overnight in the oven at 60°C. Both samples A and B were coated with platinum before being observed and analyzed via Hitachi SU8020 field emission scanning electron microscope (FESEM) [28] and Oxford X-max 50 energy dispersive X-ray (EDX).

FTIR Analysis

The observations of functional groups involved in *Rhodopseudomonas* sp. strain SBL were carried out before (sample A) and after (sample B) *Rhodopseudomonas* sp. strain SBL performed the biosorption experiments.

The dry cell biomass was prepared as previously described in the previous section. Biomass sample B will undergo biosorption experiments. Then, each biomass was dried overnight in the oven at 60°C. Both samples A and B were pretreated with KBr before being analyzed by Perkin Elmer Fourier transform infrared spectroscopy (FTIR) from 4000 to 650 cm-1.

Desorption of Cd by *Rhodopseudomonas* sp. Strain SBL

The cell biomass was prepared as previously described. The dried cell biomass was first pre-treated by resuspending the cell biomass with 1M HCl, 1M NaNO3, and 1M NaOH, respectively. Subsequently, 10 mL of cadmium solution was added into the cell, and the cell suspension underwent centrifugation at 8,000 rpm for 15 minutes. The concentration of Cd was then analyzed using Perkin Elmer AAS, and the desorption rate was calculated as below:

$$\text{Heavy metal desorption (\%)} = [C_o\text{-}C_i/ C_o] \times 100$$

Co is referred to as Cd concentration (mg/ L) before the desorption experiment, and C_i is the concentration of Cd (mg/ L) after the desorption experiment [9].

Results and Discussion

Isolation, Characterization, and Identification of PNSB

After seven days of incubation, the inoculum changes from colourless to purple, which indicates the growth of PNSB. The pure isolate shows a rod-shaped Gram-negative microbe. The biomass grew more vastly in facultative light conditions than in anaerobic conditions.

Next, the 16s rRNA sequencing results were compared with the 16s rRNA sequences in the Genbank database (NCBI). The identified PNSB was found to be 98.09% similar to *Rhodopseudomonas* sp. The PNSB was then registered under the accession number OP521893 as *Rhodopseudomonas* sp. strain SBL. Figure 1 shows the phylogenetic tree of the isolated PNSB.

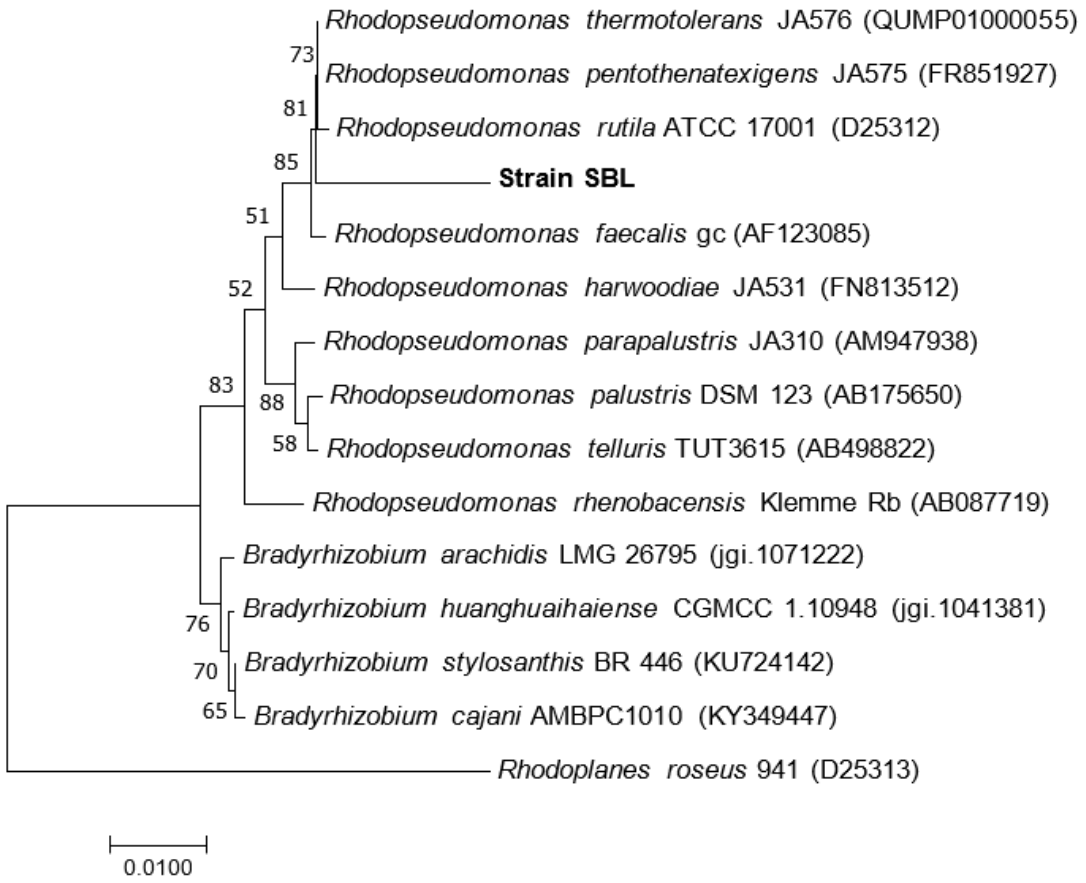

Figure 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain SBL among related *Rhodospeudomonas* species. The evolutionary history was inferred using the neighbour-joining method with bootstrap consensus tree inferred from 1000 replicates. *Rhodoplanes roseus* was used as an outgroup to root the tree

Effect of Biomass Dosage

Figure 2a shows the trendline of the data decreases when the biomass dosage increases. The 0.5 mg/ mL biomass dosage presents the highest biosorption capacity in removing cadmium ions at 76.8%. Then, 1.0 mg/ mL at 74.2%, 1.5 mg/ mL at 73.1%, 2.5 mg/ mL at 73.1%, and biomass dosage of 2.0 mg/ mL gives the lowest biosorption capacity at 72.5%.

Mohapatra *et al*., (2019) [23], mentioned that at lower biomass dosage, the quantity of accessible active binding sites is correspondingly low. Consequently, a high concentration of heavy metal left in the solution leads to decreased biomass removal efficiency. This also agrees and shows that low biomass dosage results in low biosorption efficiency. However, our study showed contradicting findings whereby biomass dosage exhibits high biosorption efficiency. Our findings are similar to those of a previous study by Li *et al*. (2023)[19], which showed that 0.5 mg/ mL absorbed the highest amount of Cd metal ions from an aqueous solution using *Sphingomonas* sp. GX_15. In addition, the decline in biosorption capacity at higher biomass dosages, as shown in Figure 2a, could result from the agglomeration of the biomass, which reduces the number of available active sites, thereby causing low biosorption capacity [7].

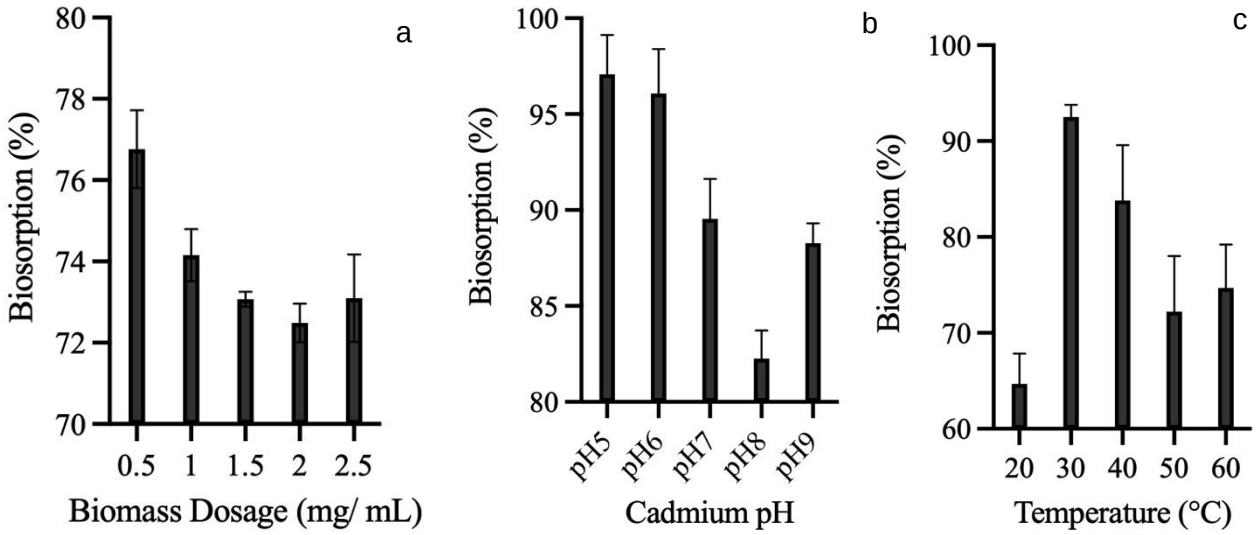

Figure 2. Biosorption capacity by non-living biomass of *Rhodopseudomonas* sp. strain SBL. (a) Effect of biomass dosage on biosorption bynon-living biomass of *Rhodopseudomonas* sp. strain SBL, (b) effect of cadmium solution's pH on biosorption by non-living biomass of *Rhodopseudomonas* sp. strain SBL and (c) effect of temperature on biosorption by non-living biomass of *Rhodopseudomonas* sp. strain SBL

Effect of Cd pH

The pH of a cadmium solution plays a crucial role in biosorption, as it affects the surface changes and functional groups on the biosorbents' active sites. Results shown in Figure 2b indicate that a pH of 5 had the most significant effect on biosorption by the non-living biomass of *Rhodopseudomonas* sp. strain SBL, achieving the highest biosorption capacity percentage of 97.1%. This was followed by pH 6, which had a biosorption capacity of 96.1%, and pH 7, which removed only 89.5% of cadmium ions. The pH levels of 8 and 9 exhibited some of the lowest removal percentages, with recorded values of 82.3% and 88.3%, respectively.

The results indicate that pH 5 exhibited the highest biosorption of cadmium by *Rhodopseudomonas* sp. strain SBL. This finding aligns with various prior studies, which have noted that maximum biosorption of cadmium ions occurs at a pH of 5 [18], [32], [37]. This is because, during low pH, there is less competition between OH^- ions and cadmium ions for binding sites [3]. Meanwhile, at higher pHs, such as pH 8 and 9, cadmium ions will be hydrolyzed into $CdOH^+$ [17], making it challenging to attach to the binding site of the cell wall. Moreover, wastewater, typically industrial wastewater containing heavy metals, is acidic [21]. Hence, the biomass in this study can potentially be a biosorbent for cadmium in wastewater due to its efficiency in biosorption ability in acidic conditions.

Effect of Incubation Temperature

As presented in Figure 2c, the most optimum incubation temperature in removing cadmium by non-living biomass of *Rhodopseudomonas* sp. strain SBL is 30°C as it removes the highest amount of cadmium ions with a biosorption percentage of 92.5%. Then, followed by 40°C, which removed 83.8% of cadmium ions. This suggests that *Rhodopseudomonas* sp. strain SBL exhibits excellent surface activity and kinetic energy at 30°C, enabling it to remove cadmium ions effectively [10]. Similarly, at 40°C, the strain retains its high removal capacity at a slightly higher temperature, proving its ability to withstand high temperatures at a certain range. At a low temperature of 20°C, the biosorption rate drops to 64.7%, the lowest among the tested temperatures. This shows that a lower temperature is insufficient to excite the surface activity and kinetic energy to remove cadmium ions, reducing biosorption efficiency [31]. As the temperature rises to 50°C and 60°C, the biosorption percentage and Cd uptake slightly reduced to 72.2% and 74.7%, 10.5 mg/ g, and 10.8 mg/g, respectively. Dixit and Singh (2013) [12] showed similar findings where the biosorption exhibited a progressive rise in the cadmium uptake as the temperature ranged from 10°C to 40°C, and a significant decrease of biosorption was observed when the temperature was beyond 40°C. The decline in efficiency at these high temperatures is attributed to the damaging effect

on the cells, including the ruptured bonds between metal ions and biosorbents, which lead to poor performance of *Rhodopseudomonas* sp. strain SBL in biosorption.

Biosorption Kinetic Models

Biosorption kinetics by non-living biomass of *Rhodopseudomonas* sp. strain SBL was investigated by applying two models, which are pseudo-first and pseudo-second-order models, to study the transportation mechanism of cadmium ions into the biosorbent [25]. Kinetics of cadmium sorption were studied for 1 ppm initial cadmium solution concentration, 0.5 mg/ mL of biomass dosage, pH 5 cadmium solution, 30°C incubation temperature, and 30 minutes contact time.

The pseudo-first order's equation was plotted for ln (qe – qt) against t (Figure 3). The K1 value was calculated from the slope of the plot while the pseudo-second-order equation was plotted for t/ qt against t. The values of qe and K1 were calculated from the slope and intercept of the graph's plot.

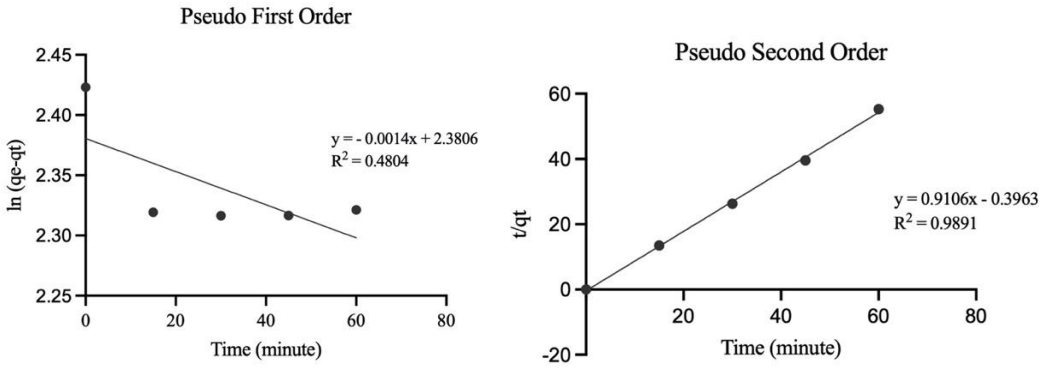

Figure 3. Pseudo-first order and pseudo-second order model by non-living *Rhodopseudomonas* sp. strain SBL biomass

A higher correlation coefficient indicates a better fit of the model for the study's adsorption analysis [6]. Figure 3 shows that the pseudo-first order's correlation factor (R^2 = 0.48) is much lower than the pseudo-second order's (R^2 = 0.99). This indicates that biosorption by non-living *Rhodopseudomonas* sp. strains SBL biomass towards cadmium removal fits the second-order model better.

The pseudo-second order model assumes the solution is heterogenous and is more applicable for a long time [30]. The pseudo-second order model is also directly linked to the available binding sites and the chemical interactions between the biosorbent and biosorbate. The rate-limiting step of the pseudo-second-order model is chemisorption or chemical sorption [24]. Chemisorption is an adsorption process involving chemical bond formation between biosorbent and biosorbent [5]. Thus, this predicts that biosorption by non-living biomass of *Rhodopseudomonas* sp. strain SBL rate-limiting step may be chemisorption. It also showed that chemical bonds were formed between *Rhodopseudomonas* sp. strain SBL and cadmium ions as the data fits into the pseudo-second order model. This data also agrees with several literature studies that reported biosorption by microbial biosorbents is often associated with pseudo-second-order models [19], [25],[24].

FESEM and EDX Analyses

Figure 4 showed the biomass of *Rhodopseudomonas* sp. strain SBL before cadmium biosorption, which portrayed visible rod shapes and appeared to have rough surfaces. According to Mohd Bahari *et al*. (2013) [24], rough surfaces provide more surface area for heavy metal biosorption, allowing cadmium ions to bind to the cell surface. As depicted in Figure 4, following the biosorption of cadmium, modifications on the cell surface took place, whereby the cell surface changed from rough to smooth. This is likely attributed to the deposition of metal on the cell surfaces. This data is similar to the observations by Mohd Bahari *et al*. (2013) [24] and Mohapatra *et al*. (2019) [23], who showed similar morphology changes before and after the biosorption of heavy metals experiments were done where the surface changed from rough to smooth. Proving that the biosorbent adsorbed heavy metal ions during the biosorption process.

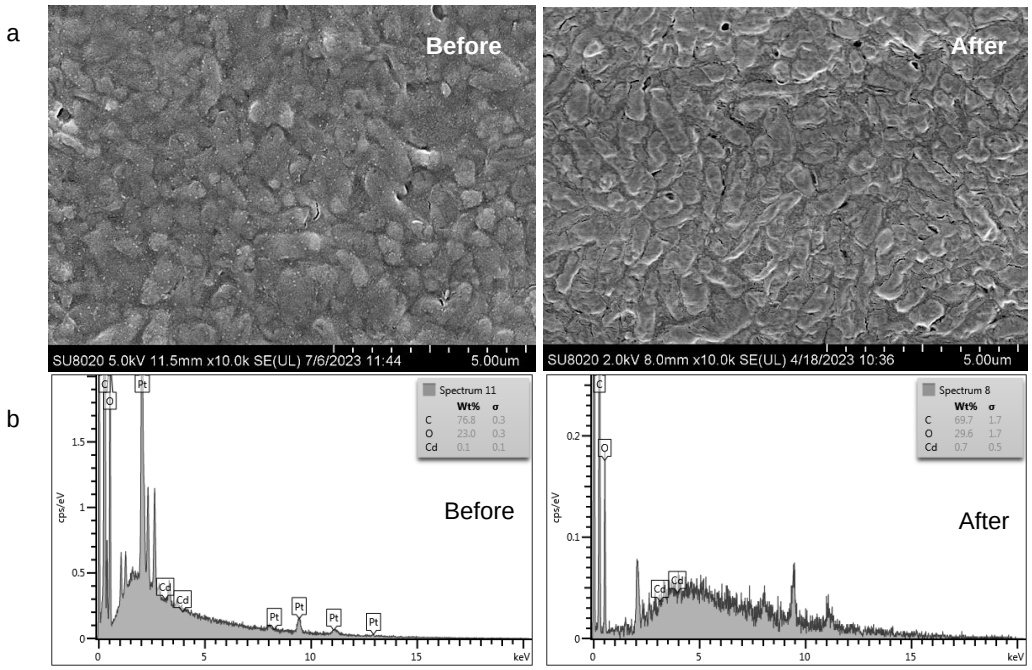

Figure 4. (a) FESEM (10 000 x) and (b) EDX analyses of non-living biomass of *Rhodopseudomonas* sp. strain SBL before and after the biosorption of cadmium

EDX analysis was performed to identify and quantify the elements present in the biosorbent. This analytical technique is crucial in characterizing the use of biosorbent and understanding their properties. According to the EDX analysis shown in Figure 4, the elemental composition of *Rhodopseudomonas* sp. strain SBL showed differences in the cadmium percentage before and after the cadmium biosorption experiment.

FTIR Analysis

FTIR spectroscopy was employed to examine the potential interactions between biomass and metal ions to identify the functional groups involved in the binding process [4], [24]. The FTIR spectral analyses revealed changes in peaks corresponding to different functional groups on the cell surface of *Rhodopseudomonas* sp. strain SBL. The FTIR analyses were performed over a broad frequency range, from 4000 cm-1 to 650 cm-1, to investigate the functional groups involved in the biosorption of cadmium by *Rhodopseudomonas* sp. strain SBL.

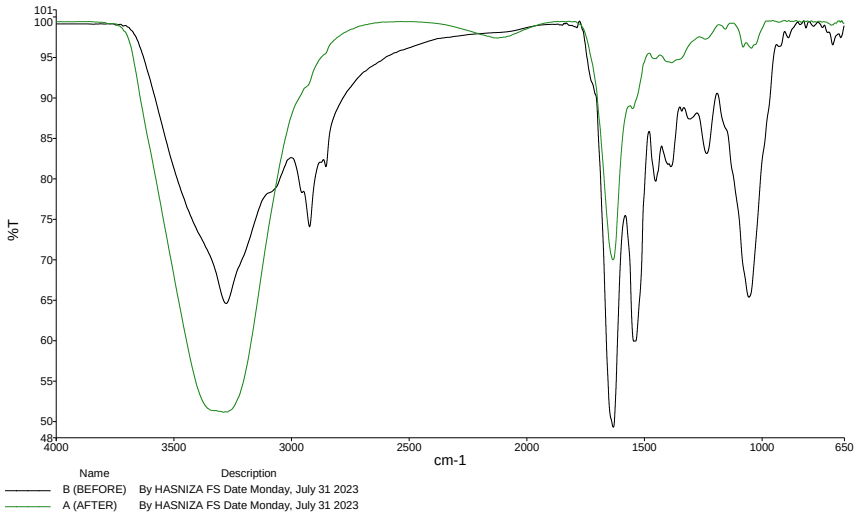

Figure 5. FTIR analysis of non-living biomass of *Rhodopseudomonas* sp. strain SBL before and after the biosorption of cadmium

The intense peak, located at 3291.74 cm-1, falls within the range of 3650 cm-1 to 3590 cm-1, indicating the presence of the O-H functional group. According to Aryal (2021) [3], this peak suggests that the lyophilized biomass contains a higher water content and has hydroxyl groups. Another peak shift is observed in the C=C stretch, moving from 1631.14 cm-1 to 1633.83 cm-1, alongside the disappearance of the C-H stretches at 2956.22 cm-1, 2923.95 cm-1, and 2854.32 cm-1. The disappearance of the C-H stretch indicates a decrease in alkyl group concentrations in the biomass. Additionally, the stretching of C=C at 1631.14 cm-1 increases to 1633.83 cm-1, while the peak for C-F decreases significantly from 1055.10 cm-1 to 1044.36 cm-1. These results indicate that hydroxyl (O-H), alkenes (C=C), and alkyl (C-F) groups primarily participate in the biosorption of *Rhodopseudomonas* sp. strain SBL.

Desorption of Cd by *Rhodopseudomonas* sp. Strain SBL

One of the most crucial aspects of biosorption studies is the reusability of the non-living biomass of *Rhodopseudomonas* sp. strain SBL. When choosing desorption eluents, the agents should be cost-effective and safe for the environment and biomass. The primary objective of this study is to recover the highest quantity of metal ions from the biosorption medium, with the recovered biomass exhibiting a strong affinity for binding metal [26].

The effective metal recovery was achieved using HCl, with 85.8%, followed by $NaNO_3$ and NaOH, with 79.5% and 78.9%, respectively, as depicted in Figure 6. Cadmium desorption occurs when the solution's pH drops below 3, making HCl one of the most effective desorption eluents. This efficacy is attributed to the abundant supply of H^+ ions, which weaken the interaction between the adsorption groups and cadmium ions. Furthermore, the Cl- ions from HCl readily form complexes with cadmium ions, facilitating their release into the solution [24].

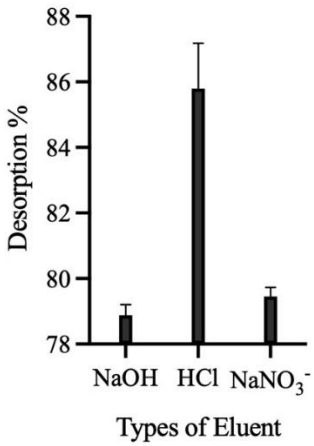

Figure 6. Desorption of non-living biomass of *Rhodopseudomonas* sp. strain SBL using different types of eluent

NaOH, on the other hand, showed the lowest desorption efficiency compared to the other two. The metal cation of interest engaged with the negatively charged functional groups on the surfaces of the biosorption, but this interaction was weaker than the others [27]. Thus, HCl is the most suitable eluent in this study, as it can recover most metals.

Based on these findings, the non-living biomass of *Rhodopseudomonas* sp. strain SBL has great potential as a microbial biosorbent. It can recover after the biosorption of cadmium with the treatment of 1M HCl. The efficiency of the non-living biomass of *Rhodopseudomonas* sp. strain SBL proves that it is cost-effective. Low maintenance would be the key advantage in choosing a biosorbent for bioremediation studies.

Conclusions

The study of using non-living biomass of *Rhodopseudomonas* sp. strain SBL as a bacterial biosorbent has provided compelling insights into its effectiveness in removing cadmium from an aqueous solution at 0.5 mg/mL of biomass, a pH of 5 for the initial Cd concentration, an incubation temperature of 30°C,

and a contact time of 30 minutes. The observed biosorption efficiency of 65% indicates a significant potential of *Rhodopseudomonas* sp. strain SBL in tackling heavy metal pollution, particularly cadmium. Additionally, the predicted rate-limiting step mechanism for *Rhodopseudomonas* sp. strain SBL is chemisorption, which aligns with the pseudo-second-order model. Furthermore, this study demonstrated the morphological changes that occurred on the cell surface of non-living biomass of *Rhodopseudomonas* sp. strain SBL after cadmium biosorption, transitioning from a rough to a smooth surface. The primary functional groups involved in cadmium biosorption by *Rhodopseudomonas* sp. strain SBL are hydroxyl (O-H), alkenes (C=C), and alkyl (C-F). A key advantage highlighted in this study is the reusability of the non-living biomass of *Rhodopseudomonas* sp. strain SBL as a bacterial biosorbent using HCl, based on the desorption studies. This capability suggests the feasibility of repeated biosorption cycles, thereby maximizing the potential of *Rhodopseudomonas* sp. strain SBL in the biosorption process. The findings indicate that the non-living biomass of *Rhodopseudomonas* sp. strain SBL is a promising bacterial biosorbent for heavy metal biosorption.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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