

# Isolation and Characterization of *Staphylococcus* spp. from a Plastic Recycling Plant and their Application in Mercury Wastewater Treatment

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## ABSTRACT

**Background and Objective:** Plastic recycling, while essential for sustainable waste management, can inadvertently lead to environmental pollution, including the release of heavy metals such as mercury, a highly toxic pollutant with serious health and ecological implications. Although *Staphylococcus* spp. are known for their diverse metabolic capabilities, their specific potential in mercury bioremediation within recycling environments remains largely unexplored. This study aims to isolate and characterize *Staphylococcus* species from a plastic recycling facility and evaluate their biosorption efficiency for mercury removal from contaminated wastewater. The influence of environmental factors, adsorption kinetics, and isotherm models was also investigated. **Materials and Methods:** Wastewater samples were collected from a local plastic recycling plant. *Staphylococcus* spp. were isolated using selective culture techniques and identified through morphological, biochemical, and molecular characterization. Batch biosorption experiments were conducted to evaluate the effects of pH (3-9), initial mercury concentration (5-50 mg/L), and contact time (up to 48 hrs). Mercury concentration was measured using atomic absorption spectrophotometry. The biosorption process was analyzed using Langmuir and Freundlich isotherms and pseudo-first- and second-order kinetic models. Regression analysis ( $R^2$ ) determined the best-fitting models. **Results:** Biosorption efficiency was highest (89.4%) at pH 7.0, with a maximum capacity of 18.9 mg/g at 25 mg/L mercury concentration. Removal peaked at 92.3% after 28 hours. The Freundlich isotherm model ( $R^2 = 0.921$ ) better described the adsorption behavior than the Langmuir model ( $R^2 = 0.571$ ), indicating multilayer adsorption. Kinetic modeling revealed the pseudo-second-order model ( $R^2 = 0.935$ ) as a better fit, suggesting chemisorption as the dominant mechanism. **Conclusion:** *Staphylococcus* spp. from plastic recycling wastewater exhibit high potential for mercury bioremediation. These findings support their application in developing eco-friendly, cost-effective treatment strategies. Future research should explore genetic mechanisms and field-scale implementation to enhance industrial applicability.

## KEYWORDS

*Staphylococci*, bioremediation, recycling, absorption, wastewater, mercury

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## INTRODUCTION

Plastic recycling offers an effective approach to reducing environmental waste, yet it also presents potential environmental and health concerns, including microbial contamination and pollutant release<sup>1</sup>. The exponential rise in plastic production has exacerbated the global plastic waste crisis, resulting in significant accumulation in landfills, oceans, and the broader environment<sup>2,3</sup>. Within recycling facilities, various microbial communities emerge due to the combination of organic waste, moisture, and fluctuating environmental conditions<sup>3</sup>. These microbes can originate from machinery, plastic materials, and environmental exposure. While some may aid recycling, others may produce harmful byproducts or pose health risks<sup>2</sup>.

*Staphylococcus*, a genus of Gram-positive cocci, is commonly found on human and animal skin and mucosal surfaces. It includes both pathogenic and non-pathogenic species. Pathogens like *Staphylococcus aureus* cause a range of diseases, whereas species such as *S. epidermidis* and *S. saprophyticus* are typically part of the normal flora<sup>4</sup>. Mercury, a highly toxic heavy metal, often enters ecosystems through mining, fossil fuel combustion, and industrial activities<sup>5</sup>. Its bioaccumulation in aquatic systems and toxicity to humans and wildlife necessitate urgent remediation strategies<sup>6</sup>.

Bioremediation utilizes microbial metabolism to detoxify or transform pollutants. Some Staphylococcal strains possess enzymes capable of degrading environmental toxins, including heavy metals like mercury<sup>7</sup>. Study reported microbial abilities to metabolize diverse chemicals for energy, highlighting bioremediation's eco-friendly potential<sup>8</sup>. However, limited data exist on isolating and characterizing Staphylococci from plastic recycling plants for mercury bioremediation<sup>9</sup>. This study addresses that gap by isolating and characterizing *Staphylococcus* strains from a recycling facility, assessing their mercury bioremediation capacity, and evaluating environmental factors influencing this ability. The study outcomes could inform environmental management, public health, and sustainable recycling practices<sup>10,11</sup>.

## MATERIALS AND METHODS

**Study area and sample collection:** The research was conducted at the Microbiology Laboratory, Federal University Wukari, Taraba State, Nigeria, over six months (January-June, 2024). Soil samples were collected from a plastic recycling plant at Ambassador Roundabout, Wukari. Samples were placed in sterile containers and transported to the laboratory within 24 hrs<sup>12</sup>.

**Materials:** Materials used included hand gloves, cotton wool, Nutrient Agar (NA), MacConkey Agar (MA), Potato Dextrose Agar (PDA), petri dishes, sterile distilled water, wire loop, test tubes, autoclave, incubator, hydrogen peroxide, and Gram staining reagents<sup>13</sup>.

**Media preparation:** Culture media (NA, MA, and PDA) were prepared following manufacturer instructions and autoclaved at 121°C for 15 min at 15 psi. After sterilization, media were cooled to 50°C before use<sup>14,15</sup>.

**Serial dilution:** Five sterile test tubes were each filled with 9 mL distilled water and arranged in a tenfold serial dilution from 10 to 10<sup>-5</sup>. One gram of soil was added to the first tube and mixed thoroughly. The 1 mL from each tube was transferred sequentially to the next and mixed<sup>16</sup>.

**Plating and incubation:** From appropriate dilutions, 1 mL was transferred to sterile petri dishes. Sterile media were poured into each dish, swirled, allowed to solidify, and incubated at 37°C for 24 hrs<sup>16</sup>.

**Purification and storage of isolates:** Distinct colonies were subcultured on fresh NA plates until pure isolates were obtained and maintained on slants at 4°C<sup>17</sup>.

## Biochemical characterization

**Gram staining:** Bacterial smears were fixed and stained using crystal violet, iodine, acetone, and safranin<sup>18</sup>.

**Catalase test:** A H<sub>2</sub>O<sub>2</sub> was dropped on a slide, and bacterial growth was emulsified. Bubble formation indicated a positive reaction<sup>19</sup>.

**Coagulase test:** Cultures were mixed with plasma in test tubes and incubated at 37°C. Clot formation indicated positivity<sup>20</sup>.

**Oxidase test:** Filter paper with bacterial growth was treated with oxidase reagent. A blue-black color within 30 sec indicated a positive result<sup>21</sup>.

**TSI test:** Isolates were inoculated into Triple Sugar Iron agar and incubated. Yellow color and/or black precipitate were interpreted<sup>22</sup>.

**Citrate test:** Simmons citrate agar was inoculated and incubated for 24 hrs. A blue color indicated citrate utilization<sup>23</sup>.

**Indole test:** Cultures in peptone water were incubated for 24 hrs. Indole reagent was added, and a red ring indicated a positive result<sup>24</sup>.

## RESULTS AND DISCUSSION

**Microbial count and colony characteristics:** Plates from 10<sup>-3</sup> to 10<sup>-5</sup> dilutions showed distinct colonies. The average viable count was 4.2×10<sup>5</sup> CFU/g on Nutrient Agar<sup>25</sup>.

**Gram reaction:** All isolates were Gram-positive cocci, appearing in clusters suggestive of *Staphylococcus* spp<sup>26</sup>.

**Catalase and coagulase test:** All isolates were catalase-positive. Two out of ten isolates were coagulase-positive<sup>27</sup>.

**Oxidase test:** Six of ten isolates developed a blue-black color within 30 sec, indicating a positive oxidase reaction<sup>28</sup>.

**Triple sugar iron (TSI) test:** Four isolates fermented multiple sugars, turning the entire medium yellow. Three showed glucose-only fermentation (yellow butt, red slant), and three produced black precipitate (H<sub>2</sub>S production)<sup>29</sup>.

**Simmons citrate test:** Five isolates turned the medium blue, indicating citrate utilization; the remainder were citrate-negative<sup>30</sup>.

**Indole test:** Three isolates developed a red ring upon reagent addition (indole-positive), while seven showed no change (indole-negative)<sup>31</sup>.

## Biosorption behavior and kinetic modeling of mercury removal by *staphylococci*

**Effect of pH:** The plot in Fig. 1 shows that mercury removal efficiency increases with pH and peaks at 89.4% at pH 7.0. This optimum is due to a balanced charge environment favoring ion exchange. Efficiency declines at extreme pH values because of proton competition and hydroxide precipitation.

**Effect of mercury concentration:** As seen in Fig. 2, increasing mercury concentration leads to higher biosorption, with uptake reaching 18.9 mg/g at 25 ppm. The trend indicates more available ions and saturation of bacterial binding sites. Lower concentrations offer less driving force for adsorption.

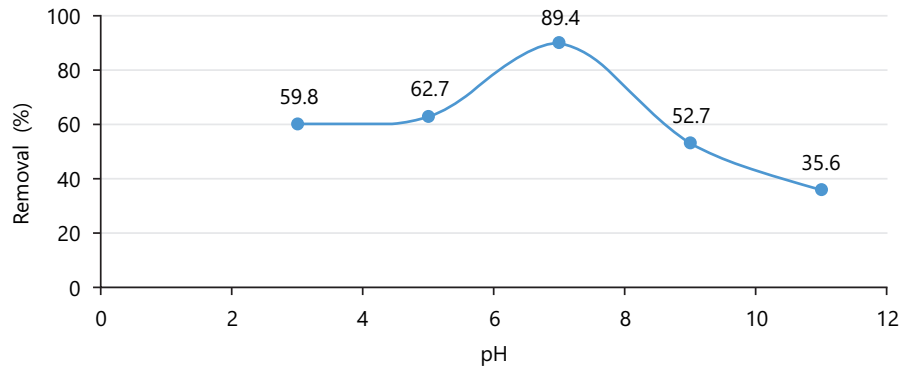


Fig. 1: Effect of pH (self-generated)

Effect of pH on mercury biosorption by *Staphylococci*, biosorption efficiency varied significantly across pH values and maximum removal (89.4%) occurred at pH 7.0 due to optimal charge interactions

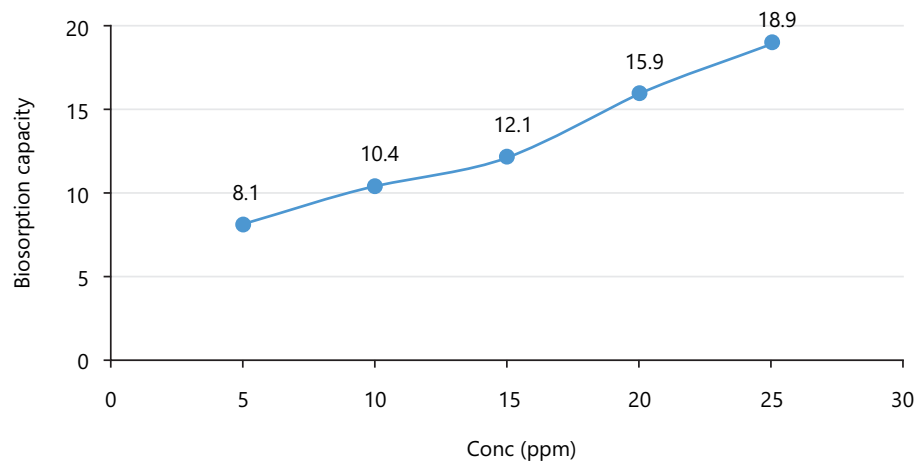


Fig. 2: Effect of Hg(II) ion concentration (self-generated)

Effect of initial Hg(II) ion concentration on biosorption capacity, biosorption capacity increased with mercury concentration up to 25 ppm and this suggests saturation of active binding sites at higher concentrations

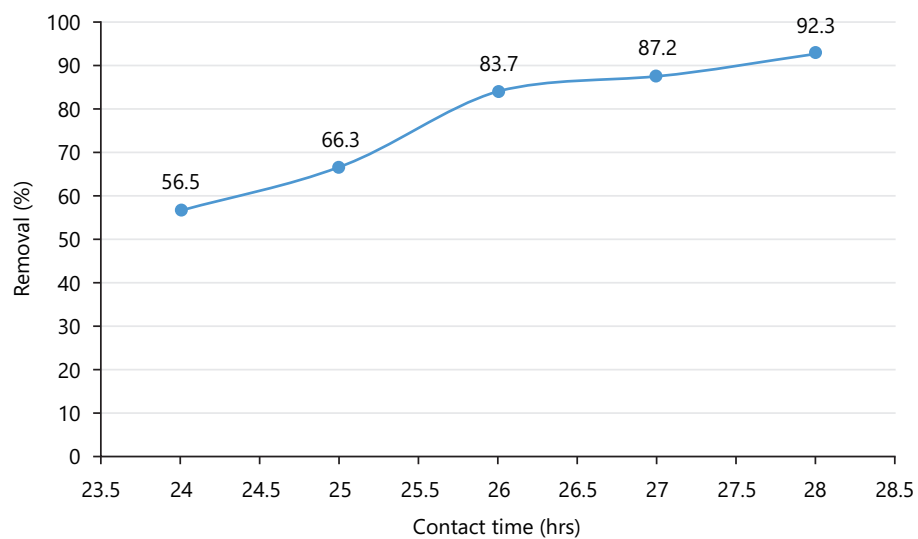


Fig. 3: Effect of time (self-generated)

Effect of contact time on mercury removal by *Staphylococci*, maximum mercury removal (92.3%) was achieved at 28 hrs and extended contact time enhanced binding to bacterial functional groups

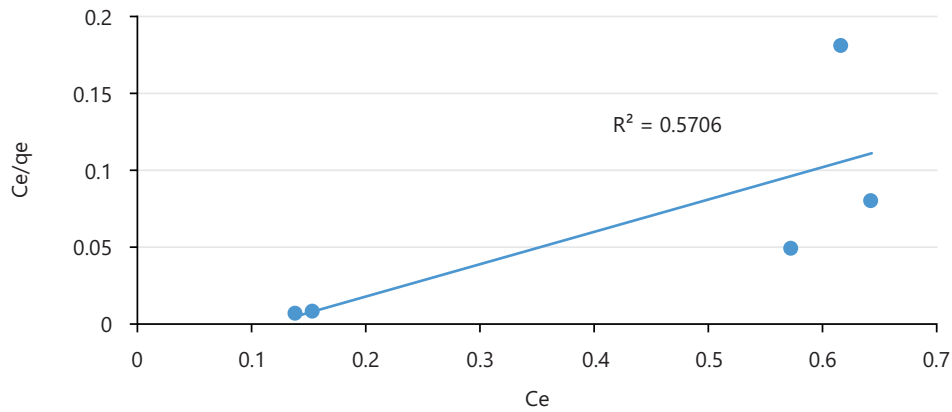


Fig. 4: Langmuir isotherm model plot for the biosorption of Hg(II) ions by *Staphylococci* isolated from a plastic recycling plant (self-generated)

Langmuir isotherm model plot for Hg(II) ion biosorption, data evaluates monolayer adsorption on a homogenous surface and Poor fit ( $R^2 = 0.571$ ) suggests non-ideal monolayer biosorption behavior

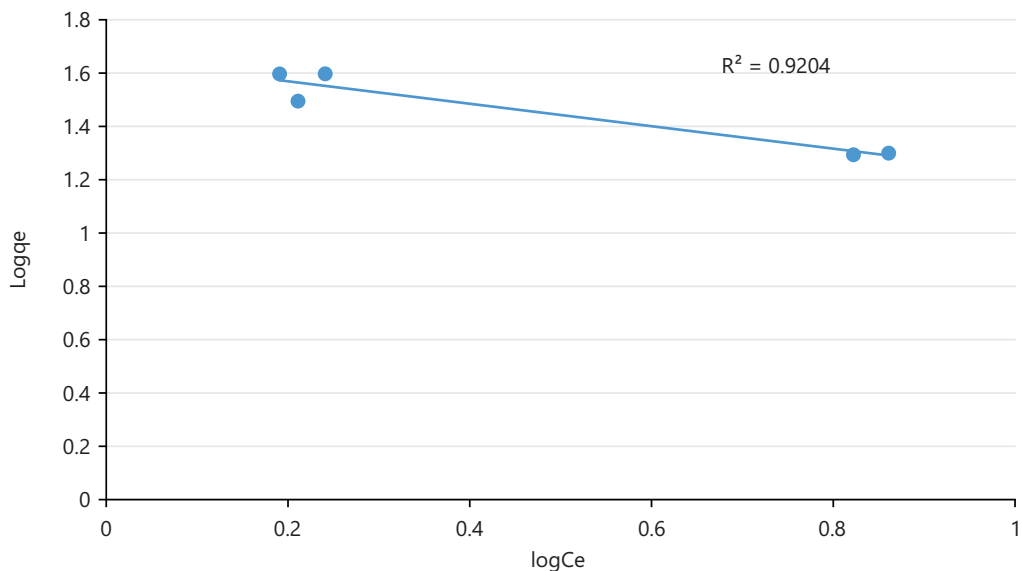


Fig. 5: Freundlich isotherm model plot for the biosorption of Hg(II) ions by *Staphylococci* isolated from a plastic recycling plant (self-generated)

Freundlich isotherm model plot for Hg(II) biosorption, model indicates multilayer adsorption on heterogeneous surfaces and best fit ( $R^2 = 0.921$ ) confirms complex surface interactions

**Effect of time:** According to Fig. 3, biosorption improves as contact time extends from 24 to 28 hrs. This increase allows more mercury ions to bind to active sites on the bacterial surface. A plateau suggests equilibrium is approached at longer durations.

**Langmuir isotherm model:** The adsorption pattern presented in Fig. 4 corresponds to the Langmuir model, which assumes monolayer adsorption on uniform surfaces. The poor fit ( $R^2 = 0.571$ ) implies that this model is less appropriate for the studied system.

**Freundlich isotherm model:** As illustrated in Fig. 5, the Freundlich isotherm describes adsorption on heterogeneous surfaces with multilayer formation. The high correlation ( $R^2 = 0.921$ ) supports the suitability of this model for mercury biosorption by *Staphylococci*.

**Pseudo-first order kinetic model:** The linearity of the graph in Fig. 6 is weak, with an  $R^2$  of 0.296, indicating poor alignment between experimental and predicted values. This suggests that the pseudo-first order model does not adequately explain the adsorption kinetics in this case.

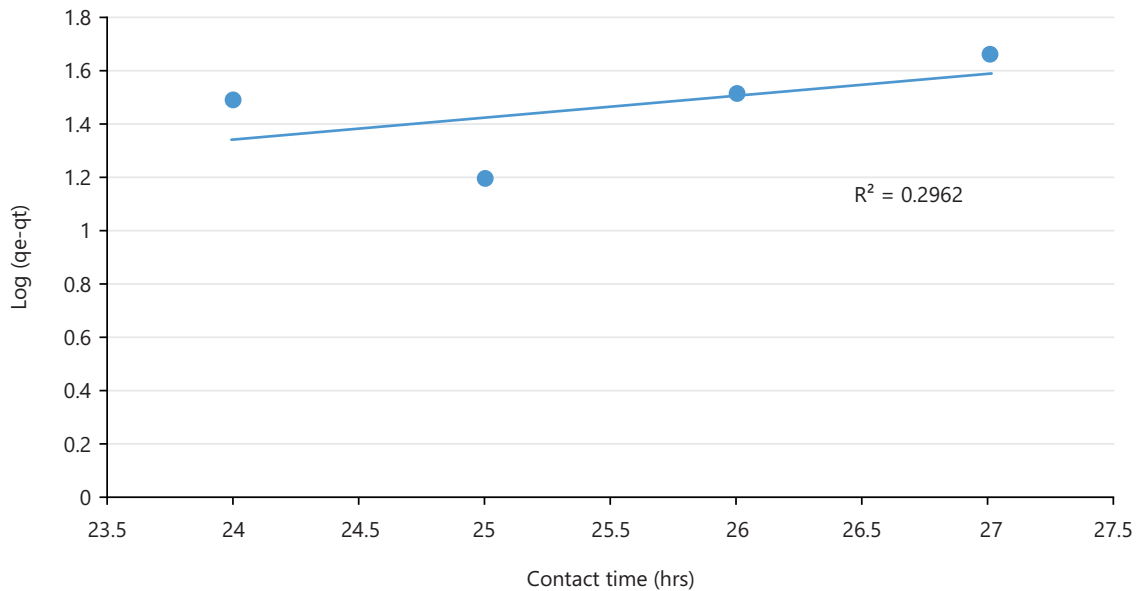


Fig. 6: Pseudo-first order model plot (self-generated)

Pseudo-first-order kinetic model for Hg(II) ion biosorption, low correlation ( $R^2 = 0.296$ ) indicates poor representation of adsorption dynamics and suggests physical adsorption may not be the dominant mechanism

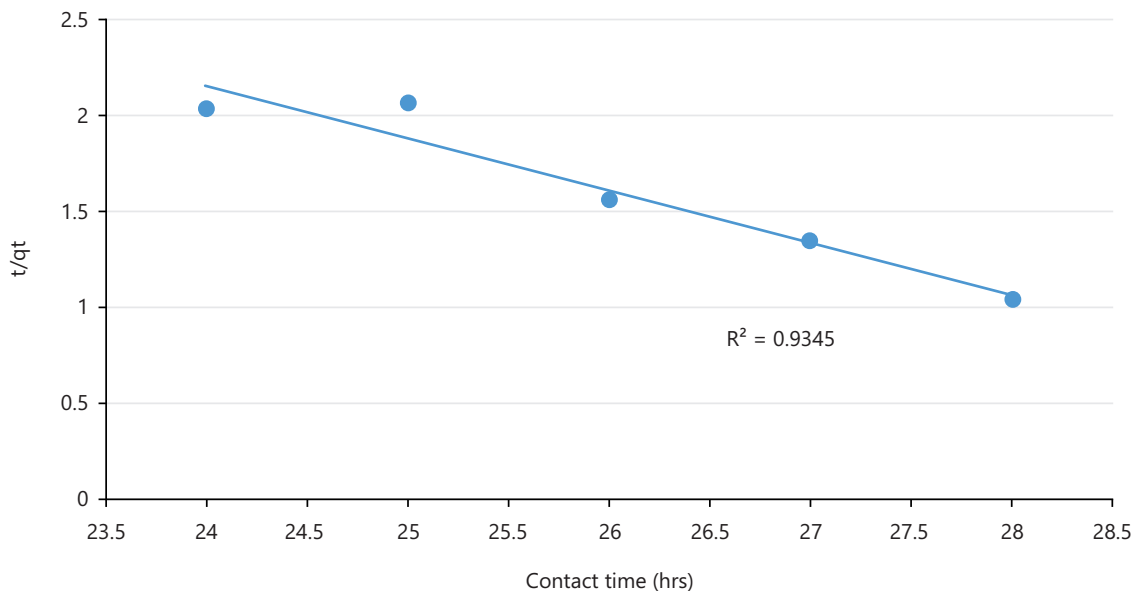


Fig. 7: Pseudo-second order model plot (self-generated)

Pseudo-second-order kinetic model for biosorption of Hg(II) ions, high correlation ( $R^2 = 0.935$ ) confirms chemisorption as the dominant mechanism and kinetics indicate active site involvement in the rate-limiting step

**Pseudo-second order kinetic model:** A stronger correlation in Fig. 7 ( $R^2 = 0.935$ ) confirms the pseudo-second order model best describes the kinetic data. This indicates that chemisorption, involving valence forces or electron sharing, governs mercury uptake by the bacteria.

**Equilibrium isotherm parameters:** Table 1 presents the equilibrium isotherm parameters for mercury biosorption by *Staphylococci* isolated from a plastic recycling plant.

- It compares the fit of the Langmuir and Freundlich isotherm models using constants and correlation coefficients
- The data highlight a better fit for the Freundlich model, indicating multilayer adsorption
- This suggests surface heterogeneity in the biosorption process<sup>32,33</sup>

Table 1: Equilibrium isotherm parameters for the biosorption of Hg(II) ions by *Staphylococci* isolated from a plastic recycling plant

Isotherm model	Hg(II)
<b>Langmuir model</b>	
$q_L$ (mg/g)	10.013
$K_L$ (L/mg)	0.110
$R^2$	0.571
<b>Freundlich model</b>	
$K_F$ (L/g)	1.02
$1/n$	0.301
$R^2$	0.921

Equilibrium isotherm constants for Hg(II) ion biosorption, Freundlich model showed higher correlation ( $R^2 = 0.921$ ) than Langmuir ( $R^2 = 0.571$ ) and data support heterogeneous, multilayer biosorption behavior

Table 2: Kinetic parameters for the biosorption of Hg(II) ions by *Staphylococci* isolated from a plastic recycling plant

Kinetic models	Hg(II)
$q_{e,exp}$ (mg/g)	28.83
<b>Pseudo-first order</b>	
$q_{e,cal}$ (mg/g)	8.214
$K_1$ (min)	0.131
$R^2$	0.296
<b>Pseudo-second order</b>	
$q_{e,cal}$ (mg/g)	25.21
$K_2$ (g/mg/min)	0.150
$h$ (mg/g/min)	1.121
$R^2$	0.935

Kinetic model parameters for Hg(II) ion biosorption, pseudo-second-order model showed superior fit ( $R^2 = 0.935$ ) and results confirm chemisorption governs the biosorption kinetics<sup>34,35</sup>

**Kinetic model parameters:** Table 2 details kinetic parameters for the biosorption of Hg(II) by *Staphylococci*.

- It compares pseudo-first-order and pseudo-second-order kinetic models using calculated adsorption capacities and correlation coefficients
- The pseudo-second-order model exhibited a higher correlation coefficient and better fit
- This indicates chemisorption as the dominant biosorption mechanism<sup>32,33</sup>

This study demonstrated that *Staphylococcus* spp., isolated from a plastic recycling facility, possess substantial potential for mercury biosorption from contaminated wastewater. The maximum removal efficiency reached 89.4% at neutral pH, with a biosorption capacity of 18.9 mg/g at a mercury concentration of 25 ppm. These results align with recent concerns raised by Singh and Walker<sup>1</sup>, who emphasized that plastic recycling, while beneficial for waste management, can also contribute to environmental contamination if pollutant mitigation is not incorporated into the system design. They advocate for integrated approaches that utilize eco-friendly, *in-situ* solutions such as microbial remediation to address pollution within recycling operations.

The role of pH in biosorption efficiency was found to be critical, with optimal mercury uptake occurring at pH 7.0. This pH likely enhances electrostatic interactions between the negatively charged bacterial surfaces and positively charged mercury ions. A similar observation was reported by Zhang *et al.*<sup>15</sup>, who explained that biosorption processes are strongly influenced by the ionization state of functional groups and the chemical form of heavy metals in solution, both of which are modulated by pH. At acidic conditions (pH 3.0), reduced biosorption was likely due to competition between protons and metal ions for binding sites, whereas at alkaline pH (9.0-11.0), mercury ions may have precipitated as hydroxides, leading to lower bioavailability for adsorption. This behavior is consistent with findings by Xia *et al.*<sup>26</sup>, who demonstrated similar pH-dependent mechanisms in their study on mercury adsorption.

An increase in initial mercury concentration led to higher biosorption capacity, though the percentage removal plateaued. This pattern suggests a saturation effect due to the finite number of active sites on bacterial surfaces. Naseem and coworkers observed a comparable trend in their study using *Sorghum bicolor* biomass, highlighting that biosorbent saturation becomes evident at elevated contaminant loads.

Contact time also influenced biosorption, with peak mercury removal (92.3%) occurring after 28 hrs. This suggests a two-phase kinetic process involving rapid initial adsorption followed by slower diffusion or chemical interactions. Such biphasic kinetics are widely reported in biosorption studies. For example, Olasehinde *et al.*<sup>35</sup> described a similar time-dependent removal pattern using *Raphia taedigera*-based activated carbon for dye biosorption. In this study, the data conformed better to the pseudo-second-order kinetic model ( $R^2 = 0.935$ ) than the pseudo-first-order model ( $R^2 = 0.296$ ), indicating chemisorption as the predominant mechanism. Bullen and collaborators<sup>32</sup> proposed an improved kinetic model sensitive to adsorbate and adsorbent concentrations, reinforcing the utility of pseudo-second-order kinetics for accurately modeling chemisorption processes.

Adsorption isotherm modeling revealed that the Freundlich model ( $R^2 = 0.921$ ) described the biosorption behavior more accurately than the Langmuir model ( $R^2 = 0.571$ ). This suggests that biosorption occurred on a heterogeneous surface with varying affinities and multilayer adsorption. Chakraborty *et al.*<sup>31</sup> previously reported similar findings in their review of microbial biosorbents, underscoring the complexity of biological surfaces rich in proteins, teichoic acids, and polysaccharides.

The effectiveness of *Staphylococcus* spp. in mercury biosorption is supported by other studies on microbial metal remediation. Aranda and Rivas, for instance, outlined the proficiency of bacterial bioadsorbents in sequestering toxic metals such as mercury and lead, emphasizing their relevance in wastewater treatment technologies. Similarly, El-Sharkawy *et al.*<sup>29</sup> demonstrated enhanced lead biosorption using *Bacillus subtilis*, which reinforces the applicability of bacterial strains for heavy metal removal under optimized conditions.

The localized sourcing of functional biosorbents from within the pollution site itself, as demonstrated in this study, supports the principle of system-specific remediation strategies. Singh and Walker<sup>1</sup> stressed the importance of such approaches within the framework of the circular economy, arguing that *in-situ* microbial solutions can reduce environmental impact and contribute to closed-loop sustainability in industrial systems.

Nonetheless, certain limitations must be acknowledged. The experiments were conducted in controlled laboratory settings that may not capture the complexity of real-world industrial wastewater, which typically contains multiple co-contaminants. Furthermore, only a single bacterial genus was explored. Future studies should investigate microbial consortia or biofilm-based systems to assess potential synergistic effects. To better understand the mechanisms involved, advanced molecular diagnostic tools such as 16S ribosomal ribonucleic acid (16S rRNA) gene sequencing, as described by Li *et al.*<sup>4</sup>, could be employed to identify biosorption-related genes and enhance strain selection through targeted genetic or metabolic engineering.

## CONCLUSION

This experimental investigation demonstrated the potential of *Staphylococcus* spp. isolated from a plastic recycling plant as an effective biosorbent for Mercury removal from aqueous solutions with a peak biosorption efficiency of 89.4% at neutral pH (7.0) and a biosorption capacity of 19.7 mg/g. Low pH reduced biosorption due to proton competition, while high pH led to mercury precipitation. The biosorption capacity also increased with increasing mercury concentrations, ranging from 8.1 mg/g at 5 ppm to 18.9 mg/g at 25 ppm. As mercury concentrations increased, the final absorbance values fell,

indicating a greater degree of mercury removal from the solution. The study reveals pH plays a significant role in biosorption efficiency. The experimental data suggested that mercury biosorption followed the Langmuir isotherm, indicating monolayer adsorption onto a homogeneous surface. The biosorption process followed pseudo-second-order kinetics, implying that chemisorption (chemical interactions) played a major role rather than just physical adsorption. These findings confirm that *Staphylococcus* spp. is a promising biosorbent for mercury remediation in wastewater treatment, particularly under neutral pH conditions, making it economical and sustainable alternative to normal mercury removal methods.

### **SIGNIFICANCE STATEMENT**

This study provides novel insights into the use of *Staphylococcus* species, isolated from a plastic recycling plant, as effective biosorbents for the remediation of mercury-contaminated wastewater. In an era where plastic recycling is both a solution to waste management and a source of environmental pollutants, this research addresses a critical intersection of microbiology, environmental biotechnology, and public health. The research demonstrates, for the first time, the capacity of indigenous Staphylococci to remove up to 92.3% of mercury under optimized conditions, with biosorption kinetics revealing a chemisorption-dominated mechanism best described by the pseudo-second-order model. The findings not only advance our understanding of microbial biosorption processes but also highlight the untapped potential of bacteria in industrial waste environments as cost-effective, sustainable, and eco-friendly agents for heavy metal remediation. This work lays the foundation for developing integrated bioremediation strategies within the framework of circular economy practices in the recycling industry.

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