

Industrial Application of Bacteria Isolated from Wukari Plastic Recycling Plant for the Bioremediation of Metal Contaminated Effluent; Isotherm and Kinetic Modelling

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ABSTRACT

Copper pollution from industrial activities poses ecological and human-health risks. This study evaluated the biosorption capacity of *Staphylococci spp.* isolated from soil at a plastic recycling facility in Wukari for removing Cu(II) from aqueous solutions. Isolates were cultured, identified by standard biochemical tests, and tested as biosorbents under varying pH, temperature, contact time, and initial metal concentration. Batch experiments determined adsorption capacity, removal efficiency, and fitted equilibrium and kinetic models. Optimal removal (86.93%) occurred at pH 7.06, 30°C, 28 h contact time, and 35 ppm initial Cu(II). Equilibrium data fitted both Langmuir and Freundlich isotherms ($R^2 = 0.988$ and 0.9676 respectively), indicating favorable adsorption onto heterogeneous surfaces with a q_{max} approximated at 25.013 mg/g. Kinetic analysis favored a pseudo-first-order model, consistent with a dominant chemisorption mechanism influenced by pH and ion concentration. The results demonstrate that *Staphylococci spp.* harvested from polluted plastic recycling environments can serve as low-cost, sustainable biosorbents for copper remediation in wastewater treatment. Practical implementation could leverage cultured microbial biomass to reduce heavy-metal loads before discharge, lowering environmental and public-health risks. The study used atomic absorption spectrophotometry to quantify residual Cu(II) and applied standard statistical fitting to validate model parameters. Collectively, these results indicate that cultured *Staphylococci* biomass can be developed into affordable biosorption materials suitable for decentralized treatment units and pre-treatment stages. Further work should validate performance in complex effluents, investigate regeneration and reuse, and explore scale-up for industrial applications.

Keywords

Bioremediation; Copper; Polluted wastewater; Recycling; *Staphylococci*

INTRODUCTION

The rapid industrialization and urbanization in recent decades have significantly increased the generation of wastewater contaminated with heavy metals (Tatah & Osagie, 2023). Among these pollutants, copper is a widely prevalent heavy metal, primarily sourced from industrial activities such as mining, electroplating, and metal finishing operations (Tatah *et al.*, 2017).

While copper is an essential trace element, its excessive presence in the environment poses severe risks to aquatic ecosystems, agricultural productivity, and human health, including liver and kidney damage, and neurological disorders. Addressing copper pollution in wastewater is therefore a pressing environmental challenge.

Bioremediation leverages the natural metabolic and biosorptive capabilities of microorganisms to remove or neutralize environmental contaminants (Abas, 2013). Compared to conventional methods, it is cost-effective, sustainable, and capable of treating low concentrations of heavy metals without producing hazardous by-products. *Staphylococci*, a group of Gram-positive bacteria commonly found in diverse environments, have demonstrated the ability to adsorb and accumulate heavy metals, making them ideal candidates for copper bioremediation (Tiwari *et al.*, 2021). Microorganisms play a crucial role in bioremediation through mechanisms such as biosorption, bioaccumulation, and enzymatic transformation. Biosorption involves the passive binding of metal ions to cell surfaces, while bioaccumulation relies on the active uptake and internal storage of metals. Enzymatic transformation, on the other hand, alters the oxidation state or chemical form of metals, reducing their toxicity and mobility (Abdel-Salam *et al.*, 2019). The efficiency of these processes depends on factors such as microbial species, metal concentration, and environmental conditions. *Staphylococci*, particularly species like *Staphylococcus aureus* and *Staphylococcus spp.* epidermidis, are known for their resilience in metal-polluted environments (Yangi & Cui, 2003). These bacteria possess unique cell wall structures rich in teichoic acids and proteins that facilitate metal ion binding. Additionally, *Staphylococci spp.* can adapt to extreme environmental conditions, including high metal concentrations, pH variations, and temperature fluctuations, enhancing their suitability for bioremediation applications (Volesky, 2007).

Traditional methods for copper removal, such as chemical precipitation, ion exchange, and membrane filtration, are often expensive and generate secondary pollution. According to Gadd (2010), bioremediation, which utilizes microorganisms to detoxify and remove pollutants, has emerged as a promising, eco-friendly alternative. Plastic recycling plants often serve as hotspots for microbial diversity due to the accumulation of various materials and pollutants, including heavy metals. These environments provide a selective pressure that fosters the growth of metal-tolerant microorganisms, such as *Staphylococci spp.* By isolating bacteria from these sites, researchers can identify strains that are pre-adapted to metal-rich conditions, thereby increasing the likelihood of successful bioremediation. This study explored the use of *Staphylococci*, a genus of bacteria known for its metal tolerance and adaptability, for the bioremediation of copper-contaminated wastewater.

MATERIALS AND METHODS

Materials & equipment

The study used standard microbiology apparatus and consumables including: atomic absorption spectrophotometer (AAS), aluminum foil, autoclave, beakers, conical flasks, digital analytical balance, pipettes, Petri dishes, masking tape, gloves, wire loop, dropper, syringe and needle, measuring cylinders, Whatman No. 1 filter paper, Potato Dextrose Agar (PDA), MacConkey Agar (MA), Nutrient Agar (NA), orbital shaker, pH meter, spatula, funnels, test tubes, mortar and pestle, and related items. These are consistent with commonly reported laboratory setups for environmental sampling, culture, biochemical testing and AAS analysis (Bhuyan *et al.*, 2023; Bankaji *et al.*, 2023; Arbefeville, Timbrook, & Garner, 2024).

Study area and period

Work was carried out at the Microbiology Laboratory, Federal University Wukari, Taraba State, Nigeria, over six months (January–June 2024). Field-to-lab transfer and cold-chain / short-term storage practices follow standard environmental microbiology procedures described in the literature (Bhuyan *et al.*, 2023; Arbefeville *et al.*, 2024).

Sample collection and transport

Soil samples were collected from the plastic recycling plant at Ambassador Roundabout, Wukari. Samples were placed into sterile containers and transported to the laboratory under aseptic conditions. Immediate processing or refrigerated storage (4 °C) until processing is standard to limit changes in viable counts (Bankaji *et al.*, 2023).

Sample Preparation and Serial dilution

For microbiological enumeration, 1.0 g of sample was suspended in 9 mL sterile diluent and mixed (yielding a 10^{-1} stock); successive 10-fold serial dilutions up to 10^{-5} were prepared by standard aseptic transfer of 1.0 mL into 9 mL sterile diluent (Bhuyan *et al.*, 2023). Aliquots (typically 1 mL for pour plate or 100 μ L for spread/spot methods) were plated on the appropriate agar (Bhuyan *et al.*, 2023; Arbefeville *et al.*, 2024).

Media preparation and sterilization

Nutrient Agar (NA), MacConkey Agar (MA), and Potato Dextrose Agar (PDA) were prepared following manufacturer instructions and standard practice (dissolve dehydrated medium in distilled water, heat to dissolve), then sterilized by autoclaving at 121 °C for 15 minutes at \approx 15 psi or following the manufacturer's validated procedure (in-house preparation and autoclave conditions are commonly used when commercial plates are not available). After autoclaving, media were cooled to approximately 50–55 °C before pouring to avoid thermal injury and ensure even pouring (Bhuyan *et al.*, 2023; Arbefeville *et al.*, 2024).

Plating, incubation and colony counting.

One milliliter (or the chosen aliquot) from each dilution was mixed with molten agar (15–20 mL poured into the plate) for pour plates or spread/spot-plated as required. Plates were incubated inverted at 37 °C for 24–48 hours and examined for colony development. For enumeration, traditional dilution plating and colony counting remain standard; more rapid microliter-spotting/micro-colony approaches (microliter spotting + micro-colony observation) have also been validated and may be used to speed counts when appropriate (Bhuyan *et al.*, 2023). Incubation conditions and times follow conventional practice and published protocols (Bhuyan *et al.*, 2023; Arbefeville *et al.*, 2024).

Isolation, purification and storage of isolates.

Distinct colonies were sub-cultured on fresh NA plates to obtain pure colonies by quadrant streaking. Pure isolates were maintained on agar slants and stored at refrigeration temperature ($\approx 4\text{ }^{\circ}\text{C}$) for short-term storage prior to further testing this approach is consistent with standard laboratory practice for maintaining isolates pending further characterization (Bankaji *et al.*, 2023; Arbefeville *et al.*, 2024).

Morphological and biochemical identification

Morphology (colony form, pigmentation, hemolysis where applicable) and cell morphology after Gram staining were used for preliminary classification. Gram staining was performed by standard smear, crystal violet \rightarrow iodine \rightarrow decolorizer (alcohol/acetone) \rightarrow safranin counterstain, with observation by oil-immersion ($100\times$). A standardized Gram-stain protocol and common pitfalls/parameters are discussed in the literature and were followed to ensure reproducibility (Li *et al.*, 2020; Arbefeville *et al.*, 2024).

Presumptive identification of *Staphylococcus* spp. was made using a panel of rapid biochemical tests commonly used in routine microbiology: catalase (3% H_2O_2 , bubbling indicates catalase-positive), coagulase (slide/tube methods to detect bound/free coagulase), oxidase (tetramethyl-*p*-phenylenediamine reagent on filter paper), triple-sugar iron (TSI) agar (stab the butt and streak the slant; incubate and record sugar fermentation/gas/ H_2S), Simmons' citrate (color change on citrate agar indicates utilization), and indole (inoculation in tryptone broth followed by Kovac's reagent; red ring indicates indole production). These conventional biochemical tests and their interpretation are reviewed in recent identification literature and remain accepted rapid tests when sequencing/MALDI-TOF is not available (Arbefeville *et al.*, 2024).

Atomic absorption spectrophotometry (AAS) for metal analysis

For any metal/Cu analyses described in the adsorption portion of the study, solid samples (if assayed by AAS) were digested using validated acid-digestion procedures (nitric acid, aqua regia, or suitable acid mixtures) chosen according to matrix and analyte; recovery and digestion method selection are critical and discussed in comparative studies (Bankaji *et al.*, 2023). After digestion/filtration (Whatman No. 1 ashless when required) and appropriate dilution, metal concentrations were measured by flame AAS or other AAS modes following instrument-specific calibration with certified standards (Bankaji *et al.*, 2023).

Adsorption isotherm modelling(Langmuir and Freundlich)

Experimental adsorption data (e.g., equilibrium concentration C_e and adsorbed amount q_e) were analyzed using standard linearized forms of the Langmuir and Freundlich isotherms to estimate q_{max} , KL , KF and n , and to evaluate model fit. The theoretical basis, typical linearization, parameter meaning and best practices for comparing isotherm models are reviewed comprehensively in recent adsorption-isotherm literature and were followed here (Mozaffari Majd *et al.*, 2022).

The study of adsorption kinetics is essential because it quantifies the rate of adsorbate uptake and thus determines the residence time of solutes at the solid-solution interface, a factor that directly governs sorption efficiency and practical system design (Al-Ghouthi, M.A. & Da'ana, D.A., 2020). It is an important characteristic in defining the efficiency of sorption (Igwe *et al.*,

2012). The data obtained from the study of adsorption dynamics are necessary to understand the variables that affect the sorption of solutes and the rate of sorption observed can also be used to develop predictive models for column experiments (Malarvizhi & Sulochana, 2008). The most important thing when searching for an appropriate sorption mechanism, therefore, is to choose a mathematical model which not only fits the data with satisfactory accuracy but also complies with a reasonable sorption mechanism (Dawodu & Akpomie, 2014).

Generally, sorption of adsorbate by an adsorbent consists of several steps which include:

- a. Transport of sorbate (solute) from the solution to the film surrounding the sorbent particles. This is called bulk diffusion.
- b. Diffusion of the sorbate from the film to the external surface (external diffusion).
- c. Diffusion from the surface to the internal sites i.e. intraparticle transport within the particle.
- d. Sorption of the sorbate on the interior surface of the sorbent (i.e. pore diffusion). This can involve several mechanisms including reaction kinetics at phase boundaries (Longmiur *et al.*, 1916; Dawodu & Akpomie, 2014)

Various kinetic models have been proposed and used to study and describe the mechanism of a Solute uptake by an adsorbent from aqueous solution (Onundi *et al.*, 2010). In this study, however, the kinetic equations employed to investigate the mechanism of metal ions adsorption are: The Pseudo-first order and the Pseudo second-order model.

RESULTS

Microbial Count and Characteristics

Several tests were carried out in order to identify and characterize microbial isolates associated with this study. Table 1 below captures the various results gotten upon subjecting the microbes to different tests and identification procedures. It was observed that most of the microbes isolated had rod shape. A few of them were found to be cocci in shape. The isolated bacterial species all tested negative to catalase. Most of the isolates tested negative to coagulase test whereas a few of them tested positive to coagulase test. All the isolates tested negative to oxidase test. Only isolates associated with the code NA 2 and NA 3 tested positive to Simon citrate test. The rest tested negative. Majority of the isolates tested positive to glucose test. Only isolates associated with codes MAC 1, NA 2, NA 3 and NA 4 tested negative to glucose test.

Table 1. Microbial count and colony characteristics

Code	Gram Staining	Catalase Test	Coagulase Test	Simon Citrate Test	Glucose Test	Sucrose Test	Galactose Test	Oxidase
MAC 1	Rod-Chain Shape	+ve	-ve	-ve	-ve	-ve	-ve	+ve
MAC 2	Cocci	+ve	-ve	-ve	+ve	-ve	-ve	-ve

MAC 3	Rod Shape	+ve	-ve	-ve	+ve	-ve	-ve	-ve
MAC 4	Rod Shape	+ve	-ve	-ve	+ve	+ve	+ve	+ve
MAC 5	Cluster Rod Shape	+ve	-ve	-ve	+ve	+ve	+ve	-ve
MAC 6	Rod Shape	+ve	+ve	-ve	-ve	-ve	-ve	-ve
MAC 7	Rod Shape	+ve	+ve	-ve	-ve	-ve	-ve	+ve
NA 1	Rod Shape	+ve	-ve	-ve	-ve	-ve	-ve	-ve
NA 2	Rod Shape	+ve	+ve	+ve	+ve	+ve	+ve	-ve
NA 3	Rod Shape	+ve	-ve	+ve	+ve	-ve	-ve	-ve
NA 4	Cocci	-ve	-ve	-ve	+ve	+ve	+ve	-ve

Biosorption Behavior and Kinetic Modelling of Mercury Removal by *Staphylococci spp.*

Effect of pH

Variations of pH are one of the most important parameters controlling uptake of heavy metals from wastewater and aqueous solutions. The effect of pH on Cu (II) removal by *Staphylococci spp.* is shown in Figure 1. These studies were conducted at an initial metal ions concentration of 7ppm in 10ml solution, at a constant time of 24hrs at room temperature, varying the pH in each solution. The percentage adsorption increases with pH. The maximum adsorption was attained at 7.06 pH and thereafter it decreases with further increase in pH. Maximum removal efficiency at optimum pH is about 58.76% metal from solution with initial Copper (II) concentration of 7ppm. This may be because at pH values below 4.20, the electrostatic force of repulsion between adsorbent (*Staphylococci*) and adsorbate Cu is prominent. At pH above 7.06, there is a possibility of adsorbate precipitation on the surface of the adsorbents by nucleation (Guatan *et al.*, 2014). According to Igwe *et al.* (2012), it was observed that increase in pH resulted to higher % removal of metal ion by groundnut husk.

According to Arivoli and Henkuzhali (2008), metal ions are more soluble in solution at lower pH values thus reducing their sorption. Hence the low sorption at low pH in this study was due

to saturation of the active sites of *Staphylococci* with hydrogen ions. However, an initial metal sorption observed with increase in pH was due to a decrease in competition between hydrogen ions and metal ions for the biosorbents surface binding sites and also due to decrease. Agraval and Singh (2007) reported a similar trend on studies of the effect of pH on the removal of Cr (VI) using *Eichhornia crassipes* root activated carbon. However, at higher pH 7 and 8 metal ions tend to precipitate out of solution. Therefore, the removal of metal ions at higher pH values is due to the formation of metal ion precipitates rather than sorption (Giri *et al.*, 2012).

Effect of Contact Time

The variation in the percentage removal of Cu (II) with contact time using pH 7.06, Cu (II) ions concentration of 21ppm, is shown in Figure 2. The maximum percent removal of Cu (II) ions is 86.93% at 28hrs and concentration of 21ppm. This was achieved by varying the contact time from 24hrs to 28hrs in separate experimental runs. As expected, the amount of copper ion adsorbed into adsorbent increases with time. The maximum removal of Cu (II) ions by *Staphylococci* was reached at 28hrs, with a maximum removal of 86.93%. This can be explained by the fact that at 28hrs there was enough time for interaction of the adsorbate and the adsorbent, also the rate of copper ion uptake was higher because all sites on the adsorbent were vacant and Copper ion concentration was high, but decrease of adsorption sites reduced the uptake rate (Kumar *et al.*, 2019). Similar results were observed in adsorption studies carried out by Kumar *et al* (2019) for the removal of Zn, Pb and Mn from aqueous medium using groundnut husk.

Effect of Adsorbate Concentration

The effect of initial adsorbate Cu (II) concentrations on the adsorption efficiency of the studied *Staphylococci* at optimum point of pH 7.06, 28hrs contact time, and temperature of 30°C is shown in Figure 3. Adsorption experiments were carried out at different initial of 7ppm, 14ppm, 21ppm, 28ppm and 35ppm. The maximum percentage removal of 86.8% was attained at a final metal ion concentration of 35ppm. These results may be explained on the basis that the increase in the number of ions competing for the available binding sites resulted in the increases in the % removal. Similar results were observed by Bose and Archana (2009) in the adsorption of Fe and Pb using *Dennettia Tripetala* which showed that the sorption capacity increased with increase in metal ion. According to Bhatti *et al.* (2017), at low ion concentrations the ratio of surface-active sites to the total metal ions in the solution is high and hence all metal ions may interact with the adsorbent and be removed from the solution. However, amount of metal adsorbed per unit weight of adsorbent (adsorption capacity) is higher at high concentrations (Oldoja *et al.*, 2012). Hence, the percent removal of heavy metals depends on the metal ions concentration and increase with increase in metal ions concentration (Onundi *et al.*, 2010).

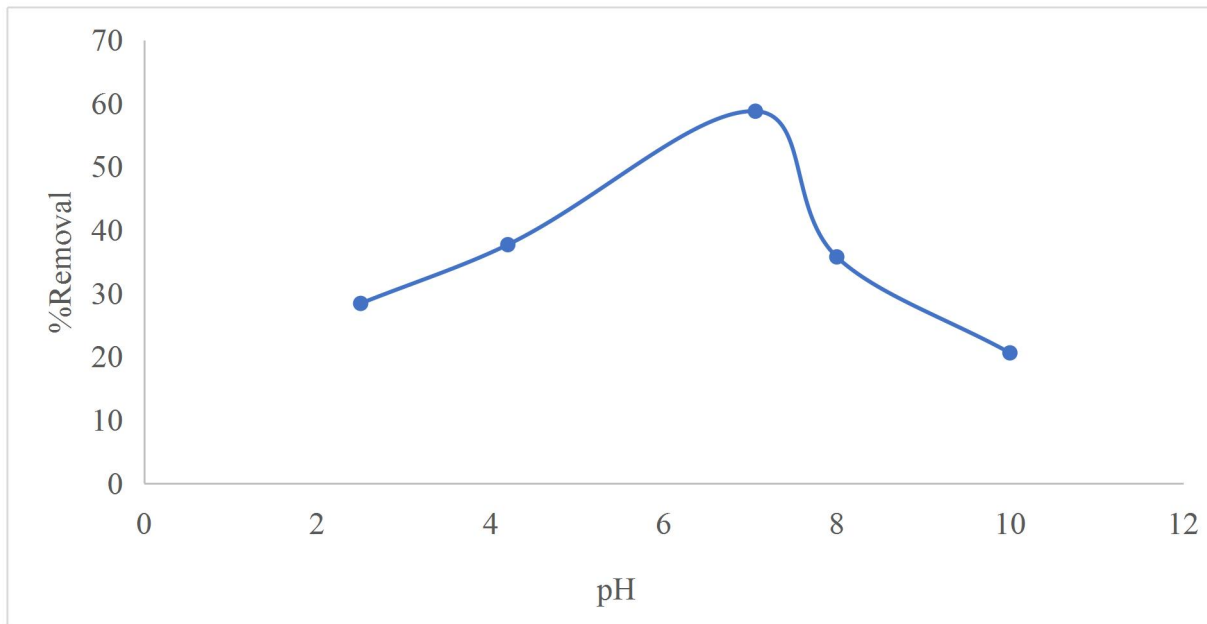


Figure 1. Effect of pH on the biosorption of Cu (II) ions by *Staphylococci spp.* isolated from a plastic recycling plant

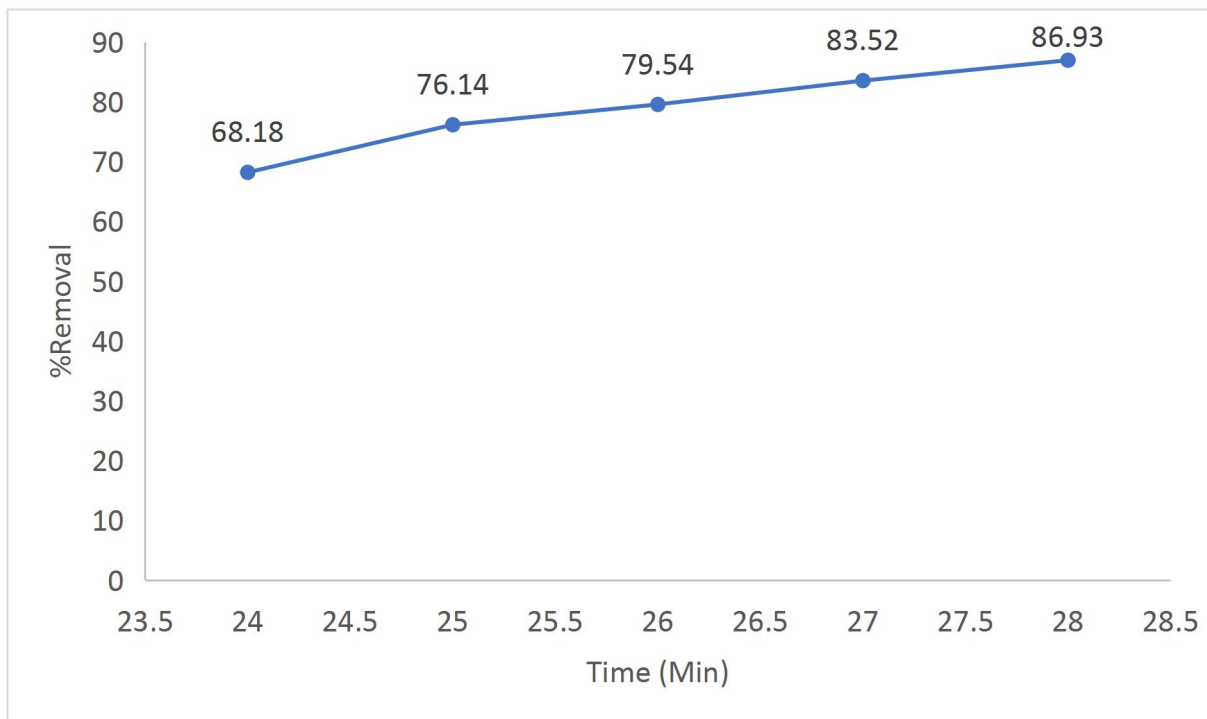


Figure. 2 Effect of contact time on the biosorption of Cu (II) ions by *Staphylococci ssp.* isolated from a plastic recycling plant

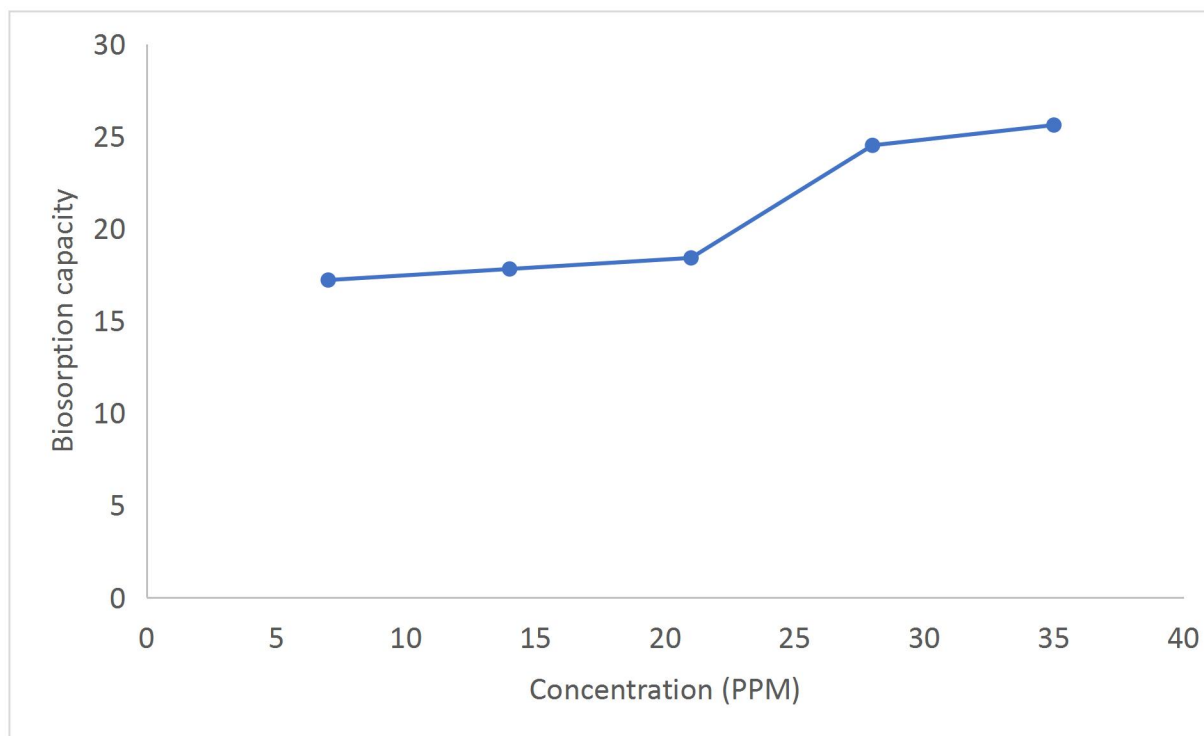


Figure. 3 Effect of Cu (II) ions concentration on the biosorption capacity of *Staphylococci* spp. isolated from a plastic recycling plant

Langmuir Isotherm

It was observed in this study that the Langmuir adsorption isotherm provided a good fit for the sorption Cu (II) ions from aqueous solutions on *Staphylococci* spp. as indicated by the high correlation coefficients (R^2) 0.988. Similar results were observed for the adsorption of Cd (II) and Pb (II) ions from aqueous solutions on *Pseudomonas* as indicated by the low correlation coefficients (R^2) 0.921 and 0.876 respectively.

Freundlich Isotherm

It was observed in this study that the Freundlich sorption isotherm provided a good fit to the experimental data compared to the Langmuir model. It was indicated by the correlation coefficient (R^2) values of 0.9676 on *Staphylococci* for Cu (II) as shown in Table 2. The value of n was observed to be (0.801) which implies that the adsorption capacity of the adsorbent and adsorption intensity is high. This confirms that the adsorbent has a heterogeneous surface since the n value does not satisfy the heterogeneity condition ($1 < n < 10$) (Flemming and Trevors, 1989). The values of KF were observed to be (1.102) implying that there was high uptake of the metal ions onto the adsorbent surface. Febrianto, (2009) also reported a high KF values for Cu (II) uptake.

Table 2. Equilibrium Isotherm Parameters for the biosorption of Cu (II) ions by *Staphylococci spp* isolated from a plastic recycling plant

Isotherm model	Cu (II)
Langmuir model	
q_L (mg/g)	25.013
K_L (L/mg)	0.310
R^2	0.988
Freundlich model	
K_F (L/g)	1.102
$1/n$	0.801
R^2	0.967

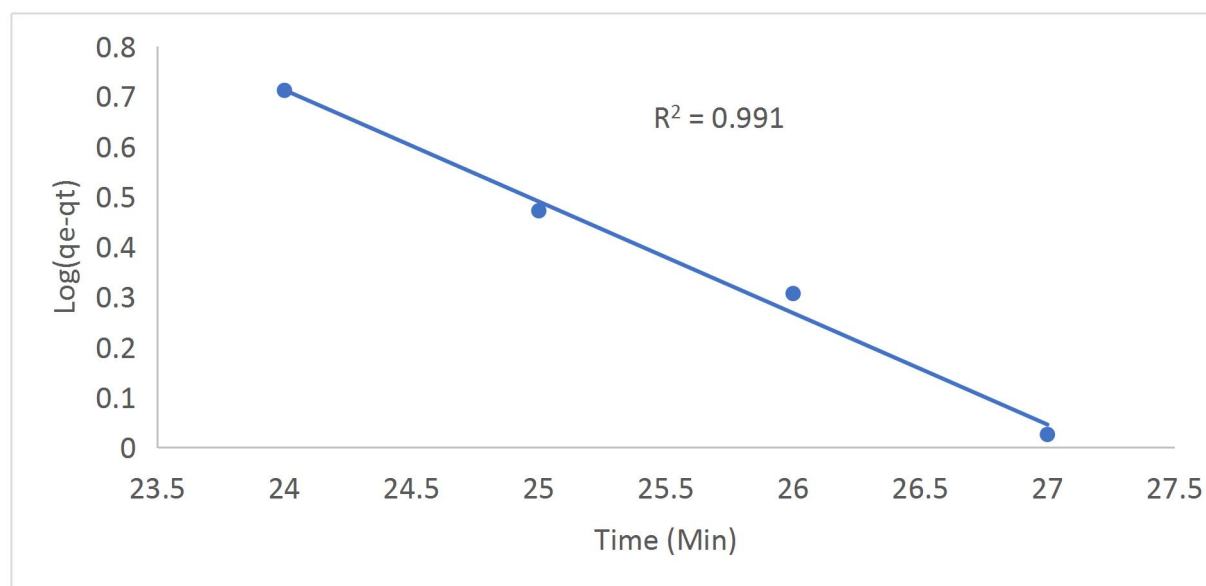
Adsorption Kinetics

The Pseudo-First Order Model

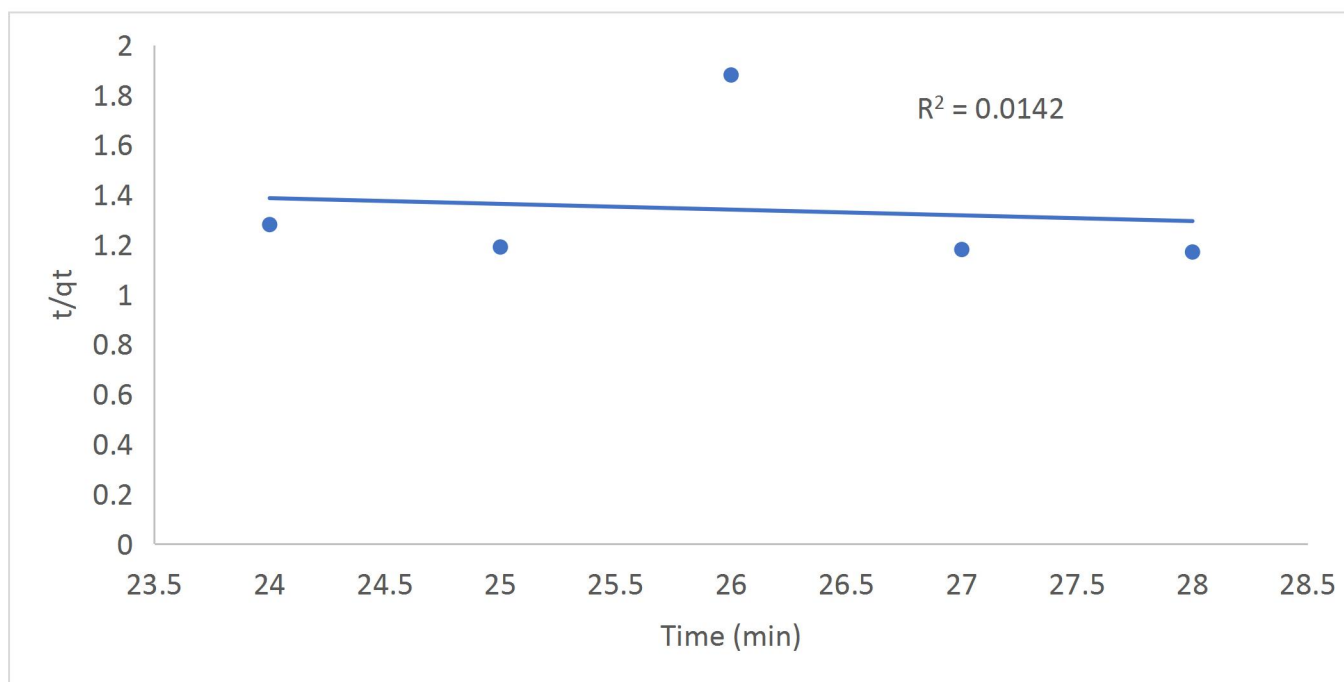
According to this study it was observed that the q_{exp} (mg/g) was 22.13 lower than q_{cal} (mg/g) which was observed to be 26.214, the k_1 (min⁻¹) was observed to be 0.031 and the R^2 value was observed to be 0.991. From this result it can be concluded that the result obtained fits the pseudo first order model indicating the validity of the pseudo first order model.

The Pseudo-Second Order Model

In this study it was observed that q_{ecal} (mg/g) was 12.209, K_2 (gmg⁻¹ min⁻¹) was 0.025, h (mg/gmin) was 1.520 and the R^2 was observed to be 0.001 which is not close to 1, indicating the invalidity of the pseudo second-order model. According to this result it can be noted that the data obtained does not fit the pseudo second-order model



Figures 4. The pseudo-first order model plot for the biosorption of Cu (II) ions by *Staphylococci spp* isolated from a plastic recycling plant. It is a plot of $\log (q_e - q_t)$ versus t derived from the pseudo-first order linear equation



Figures 5. The pseudo-second order model plot for the biosorption of Cu (II) ions by *Staphylococci spp.* isolated from a plastic recycling plant. It is a plot of $\log t/q_t$ versus t derived from the pseudo-first order linear equation.

CONCLUSION

The result of this study showed that *Staphylococci* is an efficient low-cost adsorbent for Copper Cu (II). Additionally, response surface methodology was used to investigate the interactive effect of the operating parameters namely; pH, contact time, and initial concentration of adsorbate. *Staphylococci* may be cheap and effective adsorbent for the removal Copper Cu (II) ions from waste water. Experiment results showed that maximum removal of Cu (II) ion by *Staphylococci spp.* at optimum condition (7.06 pH, temperature of 30°C, 28hrs contact time, and 35ppm concentration) is 86.93%. Based on this study, Freundlich and Langmuir isotherm provided a very good fit for the sorption Copper Cu (II) ions from aqueous solutions on *Staphylococci* as indicated by the high correlation coefficients of (R²) 0.9676 and (R²) 0.988 respectively. These experimental studies on adsorbents would be quite useful in developing an appropriate technology for the removal of heavy metal ions from contaminated industrial effluents.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest financial, personal, or professional that could have influenced the research outcomes or interpretations.

All authors confirm they have no competing interests to disclose.

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