

# POTENTIAL USE OF *STAPHYLOCOCCI SPP.* ISOLATED FROM PLASTIC RECYCLING PLANT FOR THE BIOREMEDIATION OF COPPER (II) POLLUTED WASTE-WATER; ISOTHERM AND KINETIC MODELLING

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## ABSTRACT

Copper is a major pollutant released as a byproduct during several industrial operations especially during acid mining of metal ores. Their transfer via the food web to humans is a true risk. This study aimed at investigating the efficacy of *Staphylococci* as a low-cost and eco-friendly adsorbent for the removal of Copper (II) from contaminated waste. Through series of experiments, the adsorption capacities of *Staphylococci* for Copper Cu (II) were evaluated. Soil samples were collected from a plastic recycling plant at Ambassador Roundabout, Wukari. The samples were placed in sterile containers. They were serially diluted and cultured on a nutrient agar plate. Distinct colonies were sub-cultured on fresh NA plates until pure isolates were obtained and maintained on slants at 4°C of *Staphylococci spp.* Colonies showing characteristic morphological features of *Staphylococci spp.* (smooth, pale yellow). Isolates identification was done after the subculture was carried out using some biochemical tests such as, gram staining, catalase, oxidase, coagulase, and Simon citrate and indole tests. The effect of factors such as pH, temperature, contact time, and initial metal concentration on the adsorption process was systematically examined. Findings from this study revealed that *Staphylococci* exhibits significant adsorption potential for heavy metal. Optimal removal occurs near neutral pH, with chemisorption being the dominant mechanism. Langmuir and Freundlich isotherm models provide insight into the adsorption behavior, while kinetic models highlight the process's efficiency and time dependency. These findings emphasize the potential of *Staphylococci spp.* as an effective and sustainable biosorbent for heavy metal remediation in water systems.

**Keywords:** *Staphylococci*, Bioremediation, Recycling, Polluted waste-water, Copper

## INTRODUCTION

The rapid industrialization and urbanization in recent decades have significantly increased the generation of wastewater contaminated with heavy metals (Tatah *and* 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 and 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/Apparatuses Used For the Study**

The following materials/equipment/apparatuses were used for this study: Atomic Adsorption Spectrophotometer, Aluminum foil paper, Autoclave, Beaker, Conical flasks, Digital Weight Balance, Pipette, Petri's dish, Masking tape, Hand glove, Wire loop, Dropper, Springe and Needle, Measuring cylinders, Whatman filter paper no.1, Potato dextrose Agar (PDA), MacConkey Agar

(MCA), Nutrient Agar (NA), Orbital shaker, pH meter, Spatula, Funnel, Test tubes, Mortar and pestle.

### **Study Area**

The research was conducted at the Microbiology Laboratory, Federal University Wukari, Taraba State, Nigeria, over a period of six months beginning from January, 2024 extending to June, 2024.

### **Sample Collection and Preparation**

Soil samples were collected and from a plastic recycling plant at Ambassador Roundabout, Wukari, packaged and taken to Microbiology Laboratory, Federal University Wukari, Taraba State, Nigeria. The samples were placed in sterile containers. They were then serial diluted and cultured on a nutrient agar plate.

### **Preparation of Media**

Nutrient Agar (NA), MacConkey Agar (MA), and Potato Dextrose Agar (PDA) were Prepared according to the manufacturer's instruction i.e 3.9g in 100ml and sterilized at 121°C for 15 minutes at 15 mmHg. After sterilization it was allowed to the temperature of about 55°C which is bearable by the skin.

### **Serial Dilution, Plating and Incubation**

1g of the sample was placed in a test tube and 9ml of water was added and shaken for few minutes. Five (5) test tube were arranged in ascending order  $10^{-1}$  to  $10^{-5}$  9ml of distilled water was put in each of the test tubes. 1ml was removed from the first test tube to the second was shaken thoroughly and 1ml from the second test tube to the third test tube and continued to the last test tube in that pattern. 1ml from each of the serial dilution test tube was placed in a petri plate and about 15-20ml of the sterilized agar was added mixed and allowed to solidify. Plates were incubated at 37°C for 24-48 hours in inverted positions.

### **Identification, Purification and Storage of Isolates**

Distinct colonies were sub-cultured on fresh NA plates until pure isolates were obtained and maintained on slants at 4°C of *Staphylococci spp.* Colonies showing characteristic morphological features of *Staphylococci spp.* (smooth, pale yellow). Identification after the sub culture was carried out using some biochemical test such as, gram staining, catalase, oxidase, coagulase, and Simon citrate and indole tests.

### **Biochemical Characterization of Isolates**

#### **Gram Staining**

A drop of distilled water was placed on a clean glass slide, using a sterile wire loop, little amount of the bacterial culture was spread to form a thin smear which was Air-dried and heat-fixed it gently over a Bunsen flame. The slides were stained with crystal violet and allow it to stand for 1 minute and the slides were washed gently with distilled water. Iodine solution was added and leave for 1 minute. The slides were Rinsed with distilled water and decolorize with acetone or alcohol

for 15 seconds then the counterstain with safranin for 1 minute, washed off excess stained with distilled water and air-dry Observe under a microscope using the oil immersion objective (100×).

### **Catalase Test**

A drop of 3% hydrogen peroxide was placed on a clean glass slide, using a sterile loop, small portion of the bacterial colony were transferred to the slide, mixed gently and observed for bubbling.

### **Coagulase Test**

A drop of Ajunwa's plasma was Placed on a clean slide, then a loopful of bacterial suspension to the plasma. Mixed gently and observe for clotting or gelling within 2 minutes.

### **Oxidase Test**

A filter paper was Soaked with a few drops of oxidase reagent (tetramethyl-p-phenylenediamine) then using a sterile stick or loop, a small amount of bacterial culture was smeared on the paper to observe for a color change within 10–30 seconds.

### **Triple Sugar Iron (TSI) Test**

The TSI agar butt was Stabbed with a sterile needle and streak the slant surface, was Incubated in the tube at 37°C for 24 hours. Observed for color changes and gas or hydrogen sulfide production.

### **Simmon's Citrate Test**

The surface of Simon citrate agar was streaked with the test organism, incubated at 37°C for 24–48 hours to observe for color change.

### **Indole Test**

The organism was Inoculated into tryptone broth, Incubated at 37°C for 24 hours. 5 drops of Kovac's reagent were added and observed for a red ring formation.

### **Adsorption Isotherm**

#### **Langmuir Isotherm**

Langmuir Isotherm is a monolayer type adsorption. Irving Langmuir reported the data regarding adsorption between gases and solids. The formula of isotherm as outlined below was considered: collect similar surface of adsorbent material; molecules of adsorbent material do not attract each other; uniform mechanisms of adsorption; monolayer made at the highest adsorption process and molecule of adsorbate adsorbed at the open place of adsorbent.

$$C_e/q_e = C_e/q_{\max} + 1/(b q_{\max}) \dots\dots\dots \text{equ (3.1)}$$

Where:

**q<sub>E</sub>** is the equilibrium adsorption capacity of the adsorbent (mg/g),

**C<sub>e</sub>** is the equilibrium Copper ion concentration in solution (mg/l),

**q<sub>max</sub>** is the maximum amount of metal ion that could be adsorbed on the adsorbent (mg/g) and **K<sub>L</sub>** is the Langmuir adsorption equilibrium constant.

### Freundlich Isotherm

Freundlich Isotherm model interprets the adsorption on heterogeneous surfaces with interactions occurring between the adsorbed molecules and is not restricted to the formation of a monolayer. This isotherm is commonly used to describe the adsorption of organic and inorganic compounds on a wide variety of adsorbents. The experimental data were analyzed by Freundlich Isotherm model in the linearized form:

$$\log q_e = 1/n \log C_e + \log K_F \dots\dots\dots (3.2)$$

Where:

**K<sub>F</sub>** is the Freundlich adsorption constant and it is the maximum adsorption capacity of metal ions (mg/g) and ‘n’ is the constant illustrates the adsorption intensity (dimensionless).

### Adsorption Kinetics

According to <sup>[11]</sup> the study of the adsorption kinetics of a sorption process is very important as it describes the rate of adsorbate uptake, which in turn evidently controls the residence time of the solute uptake at the solid-solution interface or the sorption reaction (Bhatti *et al.*, 2017). 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 and 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 and 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 and 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.

**The Pseudo-first order model** The pseudo-first order adsorption kinetic can be described in equ. (5.1)

$$\text{Log}(q_{\text{exp}} - q_{\text{cal}}) = \text{Log}q_{\text{exp}} - K_1/2.303t \dots\dots\dots \text{equ (5.1)}$$

Where

$q_{\text{exp}}$  is the adsorption capacity of *Staphylococci* waste in equilibrium condition (mg/g),

$q_{\text{cal}}$  is the quantity of adsorbed Copper in time (mg/g)

$K_1$  is a constant of first order equilibrium velocity (1/min).

**The Pseudo-second order model:** The second-degree adsorption kinetic model is also one of the most common models to analyze the kinetic of adsorption reactions, it is represented in equ. (5.2)

$$t/qt = 1/h + (1/q_e)t \dots\dots\dots \text{equ (5.2)}$$

where

$q_e$  (mg g<sup>-1</sup>)= Is the amount of Copper ions adsorbed at equilibrium

$qt$  (mg g<sup>-1</sup>)= Is the amount of Copper ions adsorbed at time  $t$

$h$  is described as the initial rate constant as  $t$  approaches zero.

## RESULTS AND DISCUSSION

### Microbial Count and Colony 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

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

## Biosorption Behavior and Kinetic Modeling 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 (Gin *et al.*, 2012)

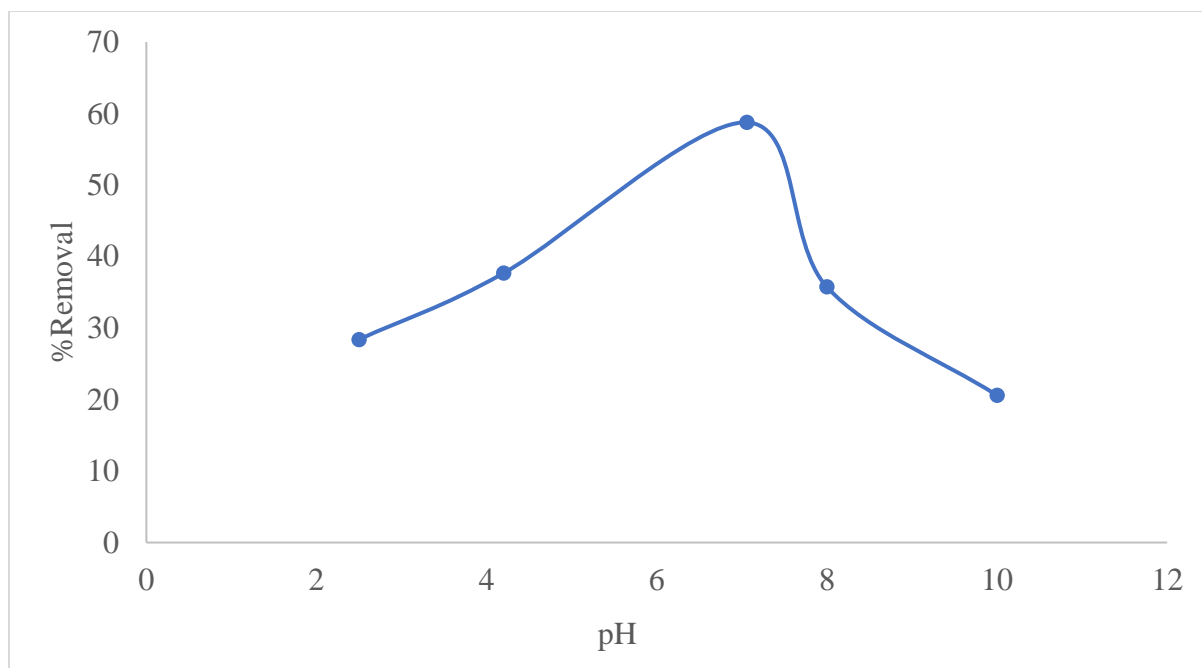
### **Effect of Contact Time**

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 s 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.

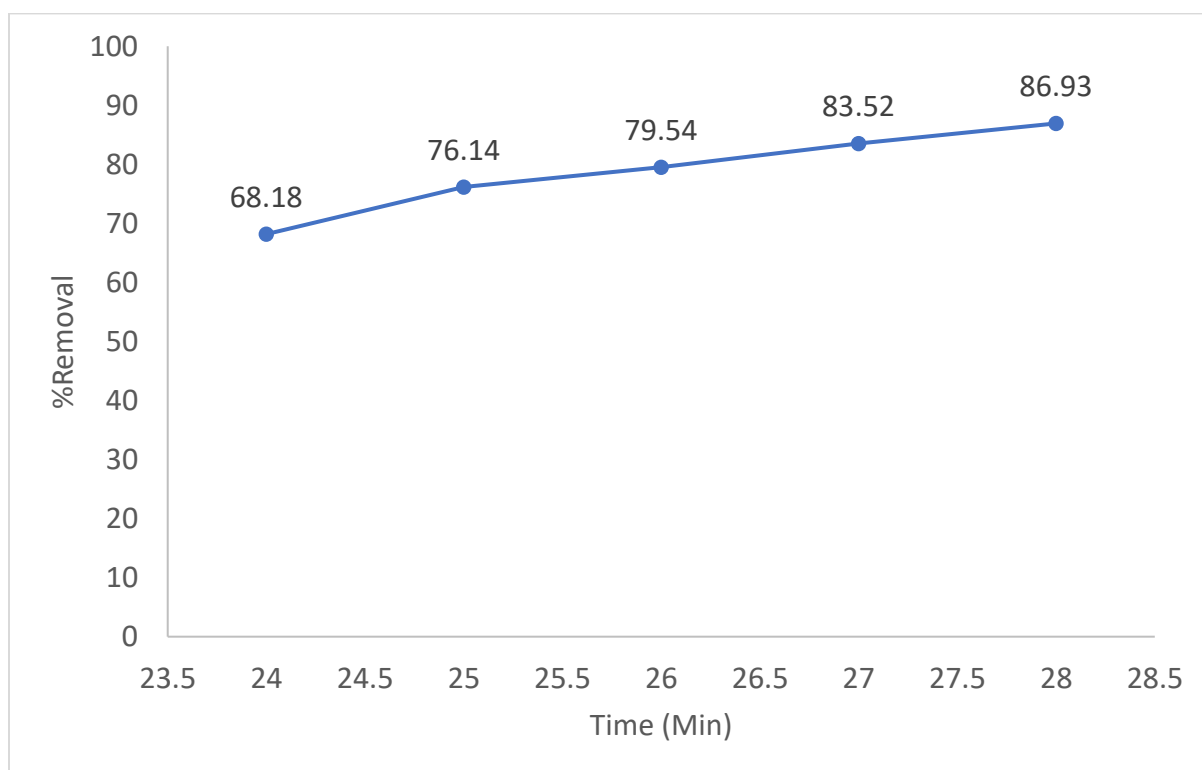
### **Effect of Adsorbate Concentration**

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. Concentration. 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).

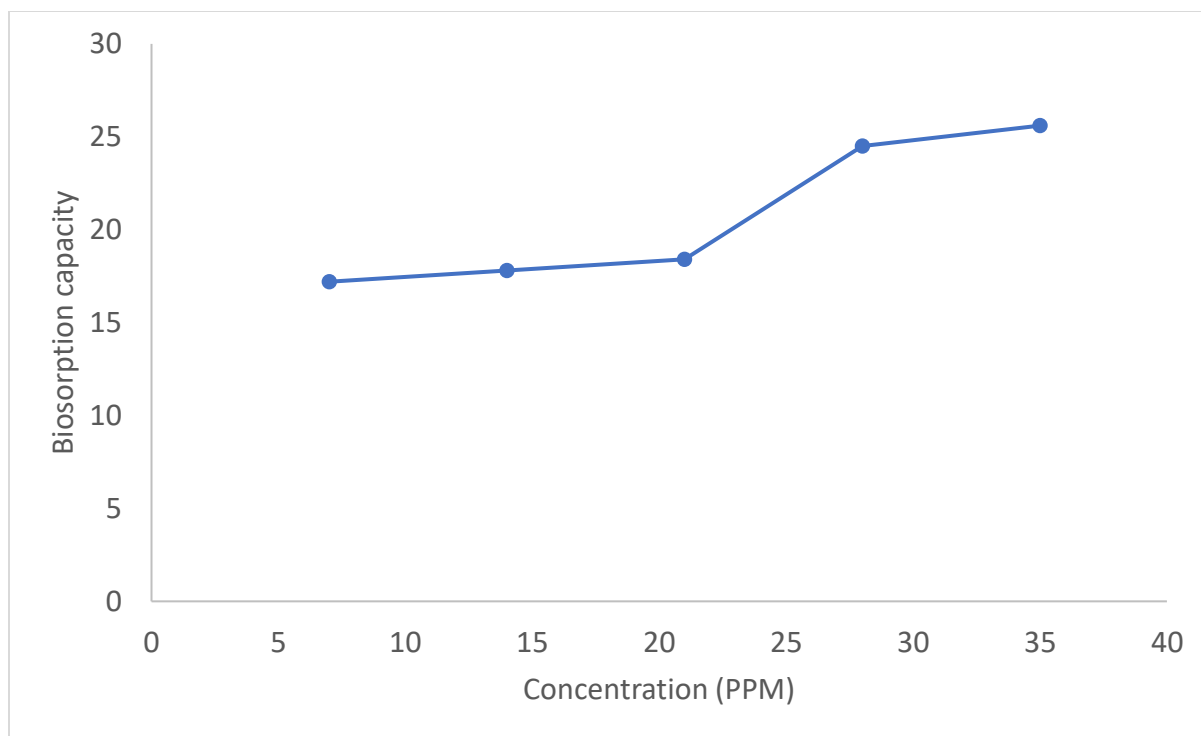




**Figure 1.** Effect of pH on the biosorption of Cu (II) ions by *Staphylococci* spp. isolated from a plastic recycling plant



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



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

## Adsorption Isotherms

### Langmuir Isotherms

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 Isotherms

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  $K_F$  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  $K_F$  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)
<b>Langmuir model</b>	
$q_L(\text{mg/g})$	25.013
$K_L (\text{L/mg})$	0.310
$R^2$	0.988
<b>Freundlich model</b>	
$K_F (\text{L/g})$	1.102
$1/n$	0.801
$R^2$	0.967

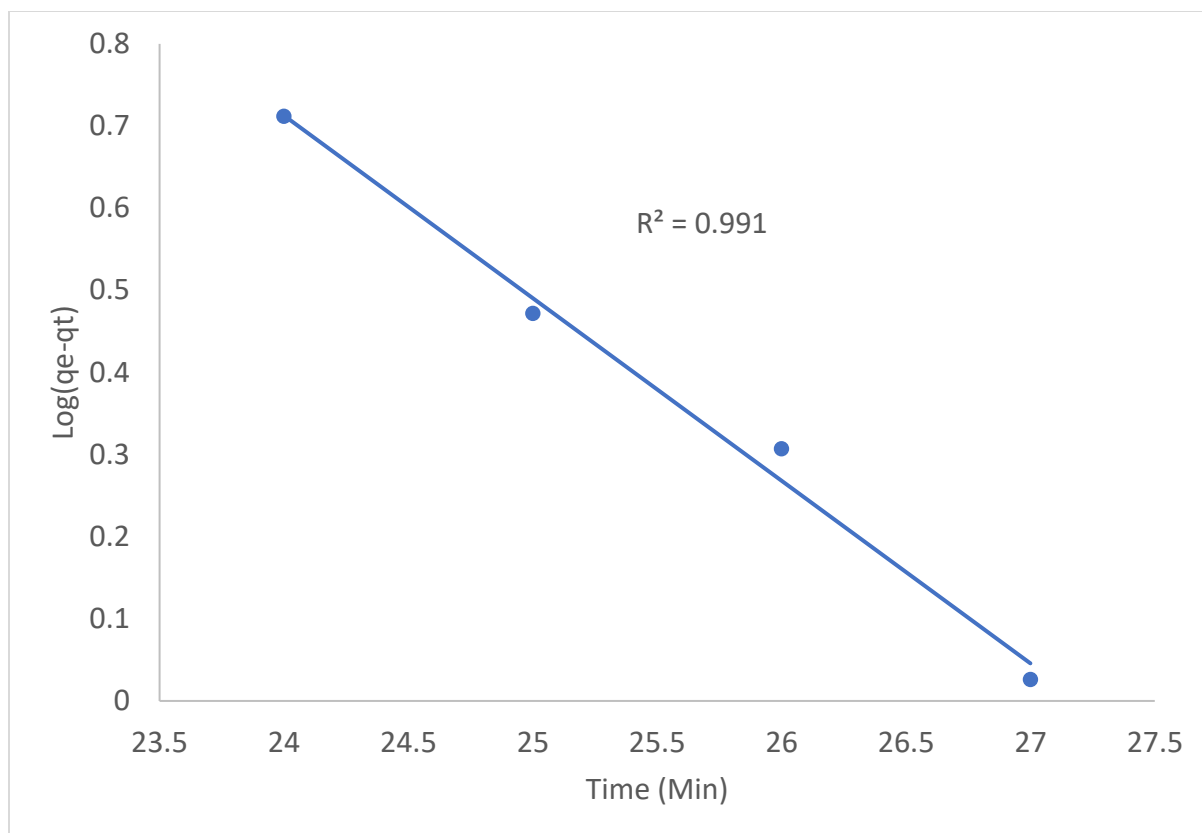
## Adsorption kinetics

### The Pseudo-First Order Model

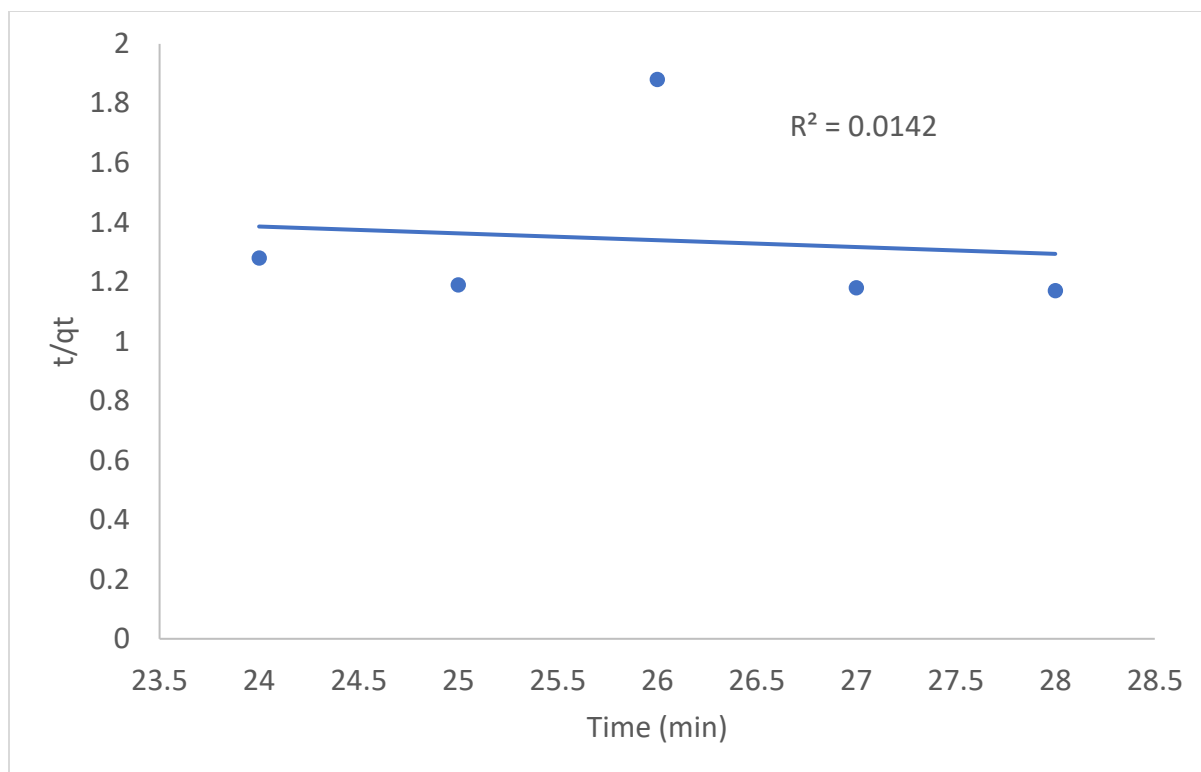
According to this study it was observed that the  $q_{\text{exp}}$  (mg/g) was 22.13 lower than  $q_{\text{cal}}$  (mg/g) which was observed to be 26.214, the  $k_1(\text{min}^{-1})$  was observed to be 0.031 and the  $R^2$  value was observed to be 0.991. From this results it can be concluded that the 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  $q_{\text{cal}}$  (mg/g) was 12.209,  $K_2 (\text{gmg}^{-1} \text{ min}^{-1})$  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  $\log t/qt$  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 (R<sup>2</sup>) 0.9676 and (R<sup>2</sup>) 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.

## CONFLICT OF INTEREST

The authors declared that there is no conflict of interest

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