BIOSORPTION OF ZINC AND MANGANESE BY *MICROCOCCUS VARIANS* AND *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SOIL

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ABSTRACT

This study was explored to examine Zinc and Manganese sorption potentials of *Micrococcus varians* and *Staphylococcus aureus* isolated from agricultural soil. The metals concentrations were determined using an Atomic Absorption Spectrophotometer (AAS). The study was carried out using 1ml of the bacterial suspension. Each suspension were inoculated into 100ml conical flasks containing 35ml nutrient broth with different concentrations of Zinc and Manganese (0.5,1.0, and 1.5ppm) respectively. The whole experiments were carried out under constant pH of 7 and temperature of 37^0C, on the basis of the factor affecting biosorption of heavy metal such as concentration, this experiment shows that the highest biosorption rate of Zinc was recorded on day 7 with 97.5% and 81.5% for *Staphylococcus aureus* and *Micrococcus varians* respectively. While the highest biosorption rate of Manganese was also recorded on day 7 with 99.2% and 99% for *Micrococcus varians* and *Staphylococcus aureus* respectively. The above result shows that; *Staphylococcus aureus* and *Micorococcus varians* has better sorption rate for Zinc and Manganese respectively. Hence, both microorganisms could be used in the removal of such metals.

1. Introduction

Effluents discharged by different industries contain metal ions which accumulate as toxic substances in the environment and pose major threats to human health as well as other living organisms (Kanu and Achi, 2011). Metals discharged into soils or water bodies are not biodegraded but undergo chemical or microbial transformations, creating large impact on the environment and public health (Volesky, 1993). Of the important metals, Hg, Pb, Cd, As and Cr (VI) are regarded as toxic while Lead, Platinum, Argon, Aurum etc, are referred to as precious metals. Uranium and Titanium are known as radionuclide (Wang and Chen, 2009). However, their extensive usage and increasing levels in the environment are of serious concern (Brown and Absanullah, 1971; Volesky, 1990). Various techniques have been employed for the treatment of heavy metal-bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies (Wierzba, 2010). However, these techniques are expensive, not environmentally friendly and usually dependent on the concentration of the waste which is ineffective in much diluted solutions (Volesky, 2001; Volesky and Naja, 2007). The search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated (Olukanni and Kokumo, 2013). Of the different biological methods, biosorption has been identified and demonstrated to possess good potential to replace conventional methods such as reverse osmosis, electro dialysis, ultra filtration, ion-exchange and chemical precipitation for the removal of heavy metals (Volesky and Holan, 1995; Malik, 2004).

The use of organisms as biosorbents is limited due to small size, operational instability and disintegration. Immobilization of biosorbents on suitable matrices which offers advantages including enhanced operational stability, ease of regeneration, increased effectiveness and re-usability have been used to solve the effects of these limitations (Volesky and Naja, 2007). However, there is the need to search for other low-cost materials that can serve as effective immobilization matrices. Biosorption therefore, refers to the accumulation of metal ions from solution by microbial or plant material. The process utilizes inexpensive dead biomass for selective sequestering of toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. Some studies have demonstrated microorganism`s ability to remove heavy metals from wastewater with better performance and lower cost (Roane *et al*., 2000). Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure. (Volesky and Holan, 1995; Modak and Natarajan, 2006). In this way, the present study was aimed at investigating the impact of *Micrococcus varians* and *Staphylococcus aureus* isolated from soil in the biosorption of Zinc and Manganese.

2. Material and methods

2.1. Collection of samples

Zinc sulphate heptahydrate and Manganese sulphate tetra hydrate was bought commercially, each compound was graded with the specification; Formula: $ZnSO_4.7H_2O$, Molar Mass: 65.37g.mol^{-1}, Density: 7.11g.cm^{-3} at 20^{0}C and melting point: 420^{0}C. $MnSO_4.H_2O$, Molar mass: 54.9380g.mol^{-1}, Density: 7.43g.cm^{-3} at 20^{0}C and melting point: 1247^{0}C. The soil sample was obtained from an agricultural site within the Federal University of Technology, Bosso Campus Minna, Niger State.

2.2. Isolation and characterization of isolates

Pour plate technique was used for the isolation of bacteria from the soil sample collected following serial dilution. The bacteria colonies that developed on nutrient agar (NA) were isolated and purified by further streaking on fresh nutrient agar. Identification of the colonies was carried out by gram staining and the well grown colonies were maintained on NA slant and stored in the refrigerator. The bacteria were characterized morphologically and identified according to Bergey's manual of bacteriology.

2.3. Metals Preparation

Stock solution was prepared by dissolving a weighed 0.883g of Zinc sulphate heptahydrate ($ZnSO_4.7H_20$) in (200ml) of d e-ionized water and 0.8111g of weighed Manganese sulphate tetrehydrate ($MnSO_4.4H_20$) dissolved in another (200ml) of de-ionized water, and then it was shook for 15minutes and allowed to stand for 24hours to obtain complete dissolution. The stock solution samples were adjusted to pH 7 by using Hydrogen chloride (HCl) and Sodium hydroxide (NaOH). Freshly grown colonies of the two isolate (*Micrococcus varians* and *Staphylococcus aureus*) were picked with a sterile wire loop, which are stirred into 10ml of an autoclaved nutrient broth which are contained in 2 different tubes respectively. The isolates were kept inside incubator for 24hours which was maintained as suspension stock for inoculation purposes.

2.4. Zinc and Manganese Biosorption Studies

One milliliter (1ml) suspensions of the isolated bacteria were inoculated into different 35ml of nutrient broth containing different concentration of Zinc and Manganese (0.5, 1.0, and 1.5ppm) each in a different samples respectively. The conical flasks were then incubated at 37^{0}C in a water bath with constant shaking so as to maintain the metabolic activities of the bacteria and The conical flasks were taken out in 7, 14, and 21day for the purpose of analysis so as to know the concentration reading of the metals biosorption. Before the analysis, the samples were centrifuge at 4000rpm for

25minutes, to obtain the supernatant, which was digested with Nitric acid (HNO_3) so as to make it clear for Analysis. This is carried out by using Atomic Absorption Spectrophotometer (AAS).

2.5. Effect of Heavy Metal Concentration

Micrococcus varians and *Staphylococcus aureus* were inoculated into 35ml of nutrient broth samples, each containing different concentrations of Zinc and Manganese respectively. The $_{P}$H of the mediums was adjusted to 7 which were incubated at 37^{0}C and heavy metals concentrations present in the digested supernatant were analyzed respectively using AAS.

Percentage biosorption was calculated using Beer Lambert's formula, which is represented below;

$$\% \text{ Biosorption} = \frac{\text{Initial metal concentration - Final metal concentration x 100}}{\text{Initial metal sorption}}$$

3. Results and discussion

3.1. Effect of Metals Concentration

Biosorption of Zinc and Manganese by *Micrococcus varians* and *Staphylococcus aureus* was studied at different concentrations (0.5, 1.0, and 1.5ppm) and at different time interval (7, 14, and 21 days). The highest biosorption was seen in *Staphylococcus aureus* on day 7 with 0.5ppm concentration of Zinc having 97.5% sorption rate while the highest biosorption of *Micrococcus varians* was also on day 7 with 0.5ppm concentration of Zinc having 81.5% as shown in (Table 1).

Table 1: Effect of Heavy Metal Concentration on Percentage Biosorption of Zinc by *Staphylococcus aureus* and *Micrococcus varians.*

Concentration of Zinc (PPM)	% Biosorption (Days)					
	Staphylococcus aureus			*Micrococcus varians*		
	7	14	21	7	14	21
0.5	97.5	73.1	71.1	81.5	74	58.2
1.0	85.1	72.6	61.7	69.5	61	50.9
1.5	82.7	57.4	55.5	68.3	58.2	46.2

The change in percentage varied in order of 0.5>1.0>1.5 concentration of Zinc (Zn), and 7>14>21 days and these changes could be as a result of Zinc at a lower concentration and a longer period could cause damage to the cell of the organisms. Munoz *et al*. (2006) reported that microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant. Thereby, they are able to sorbs the heavy metals (Prasenjit *et al*., 2005). Use of microbial resources coupled to other modern techniques is one of the most promising and economical strategies for removing environmental pollutants (Chatterjee *et al*., 2008). The result obtained on metal concentration goes in accordance with the findings of Puranik and Paknikar (2009) that increase in metal concentration beyond the optimum level in a biosorption setup results in a retardation of the process, due to the fact that the metal binding sites on the sorbent becomes saturated, leaving no space for more molecule of the metal to occupy. Horsfall *et al.* (2006) also found that increase in metal concentration above the optimum causes a reduction in the distance between the adsorbing species (the metal species), leading to the effective charge between these species becoming affected; hence the energy distribution needed for them to migrate to the binding sites on the sorbent is also disrupted.

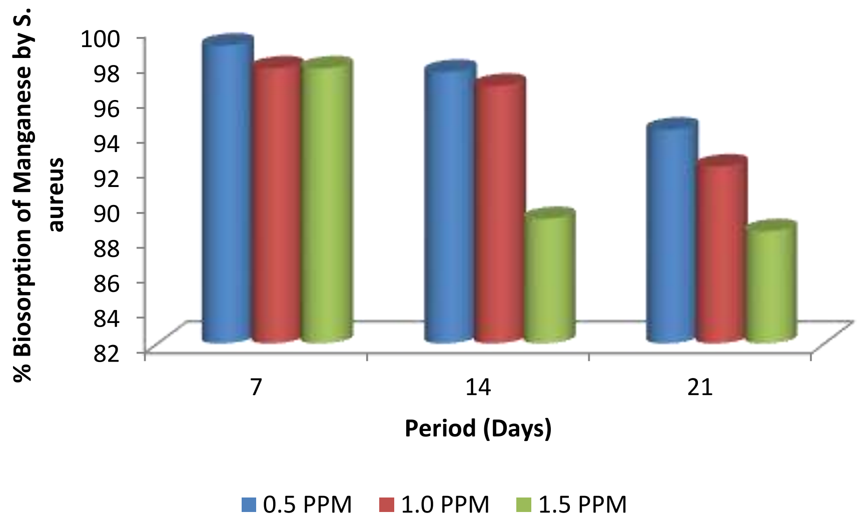

Figure 1: Percentage Biosorption of Manganese by *Staphylococcus aureus*

For the biosorption of Manganese, *Micrococcus varians* recorded the highest sorption rate on day 7 with 0.5ppm concentration of Manganese having 99.2% (Figure 2) while the highest biosorption of *Staphylococcus aureus* was also on day 7 with 0.5ppm concentration of Manganese having 99% sorption rate (Figure 1). The change in percentage biosorption varied in order 0.5>1.0>1.5ppm which implies that sorption rate decreases with increase in concentration of metal, this may be due to the availability of abundant metal species and empty metal binding sites for the bacteria to attract and remove from solution, this occurs in day 7 while in day 14 and 21 the binding sites were getting saturated (day 14) and more saturated (day 21). (Parameswari *et al*., 2009).

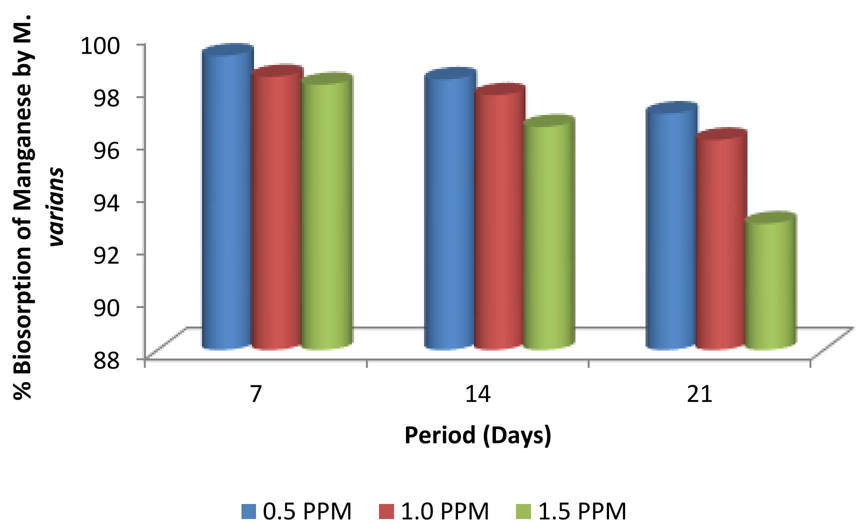

Figure 2: Percentage Biosorption of Manganese by *Micrococcus varians*

Considering the effect of metal concentration on percentage biosorption of Manganese and Zinc by *Micrococcus varians* and *Staphylococcus aureus*, the highest rate of biosorption taken from day 7 to day 21 was recorded for both metals at the concentration of 0.5ppm and the lowest rate of biosorption at 1.5ppm (Figures 1& 2). This could be as a result of the toxic effect of metals to organisms at high concentrations. The highest rates of biosorption for both metals were recorded on day 7 for 0.5ppm at 97.5% and 81.5% (Table 1) for *Staphylococcus aureus* and *Micrococcus varians* respectively for Zinc, 99% and 99.2% for *Staphylococcus aureus* and *Micrococcus varians* respectively for Manganese (Figures 1& 2). The lowest rates of biosorption were recorded on day 21 for both metals. The reason for the decline in the rate of biosorption from day 7 to day 21 could be as a result of the saturation of the organism-metal binding sites (Rai 2001). Biosorption of Zinc by *M. varians* also gave a positive result in recent work by (Kabala, *et al* 2001).

These bacteria have been earlier reported to be involved in the sorption of heavy metals from aqueous solution. (Radhi *et al*., 2012, Julian *et al*., 2 013). For biosorption of Manganese, the two bacteria have the potential of removing Manganese from solution but the one with effective sorption rate was *Micrococcus varians* with 99.2% follow by *Staphylococcus aureus* with 99% although there is no significant differences between them and two of them have high sorption rates, this might be as the result of Manganese been an essential metal and toxic only when in excess (Roane *et al*., 2000). So manganese was not toxic to *Micrococcus varians* and *Staphylococcus aureus* at those concentrations of metals.

4. Conclusion

On the basis of metal concentration with constant pH and temperature of 37^0C, this experiment showed that both organisms have the potential of sorbing Zinc but the one with high sorption rate for zinc is *Staphylococcus aureus* with 97.5% on day 7 while *Micrococcus varians* has 81.5% sorption rate while For Manganese, both bacteria almost have same sorption rate for Manganese. As the day increases, *Micrococcus varians* tends to show high sorption rate for Manganese than *Staphylococcus aureus*. Hence, *Staphylococcus aureus* could be organism in the sorption of Zinc and *Micrococcus varians* could be used for Manganese removal.

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