Abioye O.P et al (2018). Assessment of Manganese biosorption efficacy of Bacillus subtilis and Pseudomonas aeruginosa……NJTR 13(2):68-73.

Assessment of Manganese biosorption efficacy of *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated from waste dump site'

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Abstract

This study was carried out to assess Manganese removal efficiency of Bacillus subtilis and pseudomonas aeruginosa isolated from Waste Dump site. Parameters affecting the biosorption of heavy metals such as pH, Biomass Concentration, Contact time, Incubation Temperature and Initial Metal concentration were investigated. The percentage biosorption was highest on the 28 day with percentage biosorption of 78.4% for Bacillus subtilis and 80.2% for Pseudomonas aeruginosa. The optimum biosorption pH was found to be pH 5 with sorption percentage of 76.3% and 80.2% for Bacillus subtilis and Pseudomonas aeruginosa respectively. 2ml biomass concentration shows the highest sorption rate with sorption percentage of 72.4% and 65.3% for Bacillus subtilis and Pseudomonas aeruginosa respectively. When effect of temperature was taken as a parameter the sorption optimum temperature was found to be 37^{0}C with sorption percentage of 78.4% for Bacillus subtilis and 89.5% for pseudomonas aeruginosa. The sorption rate was highest at 5ppm initial metal concentration with biosorption percentage of 79.9% and 69.2% for Pseudomonas aeruginosa and Bacillus subtilis respectively. Pseudomonas aeruginosa was found to be more effective in manganese removal than Bacillus subtilis. The results obtained suggests that Pseudomonas aeruginosa possess better biosorption ability and could be used in the removal of Manganese from the environment.

Keywords: Biosorption, Manganese, pH, Incubation temperature, Contact time.
Email: bisyem2603@yahoo.com.
Received: 2016/09/14
Accepted: 2018/03/16
DOI: **https://dx.doi.org/10.4314/njtr.v13i2.11**

Introduction

Various effluents discharged by different industries contain metals 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). For more than a decade, researchers have been looking for cheaper and more effective methods to remediate heavy metal-contaminated waters and reduce the growing public health risk. Biosorption is proven to be quite effective at removing metal ions from contaminated solution in a low-cost and environment-friendly manner (Volesky *et al.*, 1990). Conventional chemical processes for metal removal include chemical precipitation, lime coagulation, ion exchange, reverse osmosis etc. These chemical methods have several disadvantages such as high processing cost and generation of toxic waste products that require disposal (Atkinson *et al.*, 1998). Alternative methods are required for the reduction of metal concentration to acceptable levels in the environment at affordable cost, therefore the use of biological method of sorption which is effective, efficient and can be industrially applied in environmental protection and metal recovery (Volesky, 1990). The use of biomass in environmental cleanup has been in practice for a while, scientists and engineers are hoping this phenomenon will provide an economical alternative for removing toxic heavy metals from industrial wastewater and aid in environmental remediation (Ahalya *et al.*, 2003). Manganese is a mineral element that is both nutritionally essential and potentially toxic. The derivation of its name from the Greek word for magic remains appropriate, because scientists are still working to understand the diverse effects of manganese deficiency and manganese toxicity in living organisms (Keen *et al.*, 1999). Manganese is a pinkish-gray, chemically active element. It is a hard metal and is very brittle. It is hard to melt, but easily oxidized. Manganese is reactive when pure, and as a powder it will burn in oxygen, it reacts with water (it rusts like iron) and dissolves in dilute acids. Manganese is essential to iron and steel production. At present steel making accounts for 85% to 90% of the total demand. Manganese is a key component of low-cost stainless steel formulations and certain widely used in aluminum alloys. Manganese dioxide is also used as a catalyst. Manganese is used to decolorize glass and make violet coloured glass. Potassium permanganate is a potent oxidizer and used as a disinfectant. Other compound that find applications are Manganese oxide (MnO) and manganese carbonate ($MnCO_3$): the first goes into

fertilizers and ceramics; the second is the starting material for making other manganese compounds. The cities of third world countries are growing at very rapid rates compared to those in the developed nations. For instance, a UN-Habitat report observed that Africa is the fastest urbanizing continent having cities like Cairo, Lagos, Nairobi, Kinshasa among others growing at fast rates that would make them triple their current sizes by the year 2050 (UN-Habitat, 2010). Such high rate of growth of cities has implications for the provision of urban infrastructural services to prevent the proliferation of urban slum. The increasing growth of cities, therefore, has implications for municipal waste management among other social services required in the urban communities. Data from many of the cities shows inadequacy in urban social services like shelter, provision of safe drinking water and efficient management of solid wastes. The cities are therefore littered with 'mountains' of rubbish in landfills and open (in most cases illegal) waste dumps which are covered with flies and thus serve as breeding grounds for rodents and mosquitoes which are carriers of diseases. Studies in children have suggested that extremely high levels of manganese exposure may produce undesirable effects on brain development, including changes in behavior and decreases in the ability to learn and remember. In some cases, these same manganese exposure levels have been suspected of causing severe symptoms of manganism disease (including difficulty with speech and walking). We do not know for certain that these changes were caused by manganese alone. We do not know if these changes are temporary or permanent. We do not know whether children are more sensitive than adults to the effects of manganese, but there is some indication from experiments in laboratory animals that they may be.

Biosorption which is the process of passive ion binding by dead or living biomass represents a cost- effective way of eliminating toxic heavy metals found in the environment (Kratochvil and Volesky, 1998). It is preferred to chemical methods due to absence of toxicity constraints, effective metal recovery amongst others. Different bacteria, fungi and seaweeds species used as biosorbents in the removal of manganese, aluminium and other metals from wastewater or solution has been reported (Wang and Chen, 2009). Several studies related to wastewater treatment were carried out using low-cost materials such as: rice husk (RH) , that has interesting properties such as hydrophilic, porous, and high surface area, is promised as adsorbent (Pehlivan *et al.*, 2013).

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, 2005). Hydrogels such as polyacrylamide, gelatine, calcium alginate and k-carrageenan have also been used as immobilization matrices (Sharanagouda and Karegoudar, 2002; Adhinarayana *et al.*, 2005; Ivanova *et al.*, 2010; Usha *et al.*, 2010; Vijayanand *et al.*, 2012). However, there is the need to search for other low-cost materials that can serve as effective immobilization matrices. The aim of this study is to assess biosorption of manganese by bacteria isolated from waste dump site.

Materials and methods.

Sampling and screening of bacterial Species.

Soil sample from Maikunkele solid waste dumpsite was collected in a sterile container and transported to the laboratory for screening. One gram of the soil sample was diluted in 9ml of sterile distilled water and was mixed for 15minute, 1 ml of the serially diluted samples was plated on nutrient agar plate by pour plate method and incubated at 37^0C for 24hours. The colonies were counted and expressed as a colony forming unit per gram (cfu/g). The colonies were sub-cultured repeatedly on nutrient agar to get a pure culture.

Characterization and Identification of Microbial Isolates

Characterization of bacterial isolates was based on Gram staining, colonial morphology and biochemical tests. The biochemical tests carried out include: production of catalase, oxidase, coagulase, citrate utilization, starch hydrolysis, indole, hydrogen sulphide production. The bacterial isolates were identified by comparing their characteristics with those of known taxa using the schemes of Brener *et al.* (2005).

Metal Solution Preparation

Stock solution (500ml) was prepared by dissolving 0.5g of potassium per manganate (KMnO2) in deionised H_2O, shaking it for 15 minutes and then leaving it to stand for 24 hours to obtain complete dissolution. Solutions were adjusted to desired pH values with 0.1m Sodium hydroxide. The initial Manganese concentration was measured at the beginning of all experiment carried out using Atomic Absorption Spectrophotometer (AAS), Accusys 211, Buck scientific, USA.

Determination of metal concentration.

The heavy metal concentration was determined by the use of atomic absorption spectrophotometer. Determination of manganese was done by using its specific lamp and at a specific wavelength. The percentage removal of manganese was calculated according to the following equation:

$$\% \text{ Removal of Mg ion (\% R)} = \frac{C_1 - C_2}{C_1} \times 100$$

Where C_1 and C_2 are the initial and final concentration (mg/L), respectively

Biosorption Experiments.

Fifty millilitres (50 ml) of nutrient broth was prepared in 50 ml Erlenmeyer flasks and sterilized at 121^{0}C at 15 psi pressure for 20 minutes. Two millilitres of fresh inoculum was inoculated into 50 ml of nutrient broth and various concentrations of metal stock solutions were added to each flask to attain different initial metal concentration. These experimental flasks with cultures were incubated at 37^{0}C for 28days. Samples were drawn on 7, 14, 21 and 28 days and the supernatant and cells were separated by centrifugation at 4000 rpm for 25 minutes. The biomass and the broth were digested separately with aqua regia (HCl: HNO3 at 3:1 ratio). Bulk Scientist Atomic Adsorption Spectrophotometer (AAS), was used for Manganese estimation. All experiments were conducted in triplicates and the biosorption studies was done using biomass as a function of various parameters such as pH, biomass concentration, temperature, time and initial metal concentration

Results and Discussion

The bacteria isolated from waste dumpsite were identified as *Bacillus subtilis* and *Pseudomonas aeruginosa.*

Effect of Metal Concentration.

The initial metal concentration was observed to have a profound effect on the biosorption rate. Metal removal by bacteria was rapid at the initial time of the biosorption with a sharp increase between 7 and 14 days. However Between 21 and 28 days the sorption rate was only marginal which could be due to saturation of binding site. Initial metal concentration of 5ppm has the highest sorption rate. This implies that sorption rate decrease with increasing concentration of metals. *Pseudomonas aeruginosa* was effective in manganese removal up to 79.9%, 65.4% and 61.3% for initial metal concentration of 5ppm, 10ppm, and 15ppm respectively. *Bacillus subtilis* was effective in manganese removal up to 69.2%, 60.2% and 59.3% for initial metal concentration of 5ppm, 10ppm, and 15 ppm respectively at the end of 28 days. The percentage of metal removal increased with time and reached a maximum at equilibrium time after which the reduction rate remained constant.

Pseudomonas aeruginosa has a higher uptake of manganese than *Bacillus subtilis*. Table 1 illustrates the effect of initial metal concentration on manganese biosorption using *Pseudomonas aeruginosa* and *Bacillus subtilis.*

Table 1 Effect of metal concentration on manganese biosorption by *Pseudomona aeruginosa* and *Bacillus subtilis*.

Concentration of Manganese(PPM)	%Biosorption(Days)							
	Pseudomonas aerugenosa				Bacillus subtilis			
	7	14	21	28	7	14	21	28
5	50.0	76.3	78.9	79.9	47.3	60.0	68.8	69.2
10	45.9	57.5	64.6	65.4	40.35	54.3	66.6	60.2
15	44.8	55.1	61.3	62.0	37.9	50.3	58.6	59.3

Effect of pH

Fig. 1 illustrates the effect of pH on sorption of manganese by *Pseudomonas aeruginosa* and *Bacillus subtilis*. This was studied over a range of pH 1 to 9. Biosorption of metals is pH dependent. The percentage metal sorption varies with pH of the medium. The percentage sorption was highest at pH 5 for both *Pseudomonas aeruginosa* and *Bacillus subtilis* with percentage sorption of 80.2% and 76.3% respectively hence the optimum pH for manganese biosorption was found to be 5. The percentage biosorption increases from pH 1 to 5 and starts to decrease from pH 6 to 9. The fluctuation beyond pH 5 could be due to decrease of low availability of surface for sorption at low pH and formation of metal hydroxide and other metal-ligand complexes which significantly reduce the amount of metal ions sorbed at high pH (Vijayaraghavan and Yun, 2008).

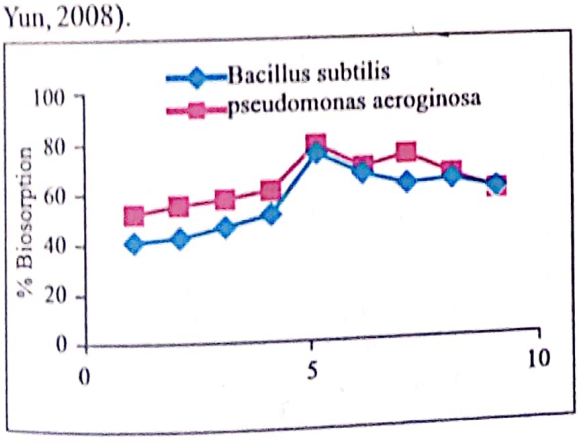

Figure 1 Effect of pH on percentage Manganese biosorption by *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Effect of Biomass Concentration

Biosorption of manganese was studied at varying biomass concentration of 0.5ml, 1ml, 1.5ml and 2ml. The sorption increases with increasing biomass concentration and was highest at biomass concentration of 2ml with percentage biosorption of 65.3% and 72.4% for *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively and least at 0.5ml biomass concentration with percentage biosorption of 54.3 and 35.4 for *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. This may be due to availability of enough binding site in 2ml biomass concentration than in 0.5ml biomass concentration (Fig. 2).

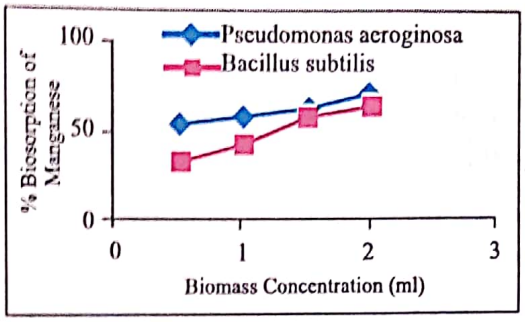

Figure 2 Effect of biomass concentration on percentage Manganese biosorption by *Pseudomonas aeruginosa* and *Bacillus subtilis*

Effect of Temperature.

The biosorption study was carried out at two different temperatures: 25°C and 37°C representing room temperature and bacteria incubation temperature respectively. The sorption rate was highest at temperature 37°C with percentage biosorption of 89.5% and 78.4% for *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively while the percentage biosorption was 69.7% and 58.3% at temperature of 25°C for *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively (Figure 3) Higher sorption rate at temperature 37°C than at 25°C may be due to shrinkage of cells at lower temperatures which reduces the surface area of contact (Vijayaraghavan and Yun, 2008).

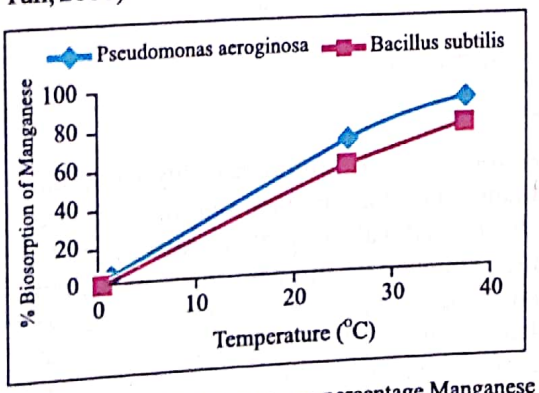

Figure 3 Effect of temperature on percentage Manganese biosorption using *Pseudomonas aeruginosa* and *Bacillus subtilis*

Effect of Contact Time.

The biosorption rate increased with time. This explains the effect of contact time on sorption process. Metal removal by bacteria was rapid at the initial days of the biosorption with a sharp increase between day 7 and 14. However, between 21 and 28 days the sorption rate was only marginal which was almost constant due to the saturation of the binding site. On the 7th day the sorption percentage was 43% for *Bacillus subtilis* and 48% for *Pseudomonas aeruginosa* which increased rapidly up to 65% for *Bacillus subtilis* and 70.2 for *Pseudomonas aeruginosa* on the 14th day. The sorption rate became gradual after 14 days with percentage sorption of 73.5% and 78.4 % for *Bacillus subtilis*, and 74.4% and 80.2% for *Pseudomonas aeruginosa* on days 21 and 28 respectively. Hence there is no significant difference between 21 and 28 days (Fig. 4). Parameswari *et al.* (2009) explained that the rate of absorption of metals is in two phases, an initial phase of faster absorption followed by the phase of slower absorption. The initial faster uptake might be due to the availability of abundant metal species and empty metal binding sites of the microbes. The slower phase might be due to saturation of metal binding sites (Parameswari *et al.*, 2009).

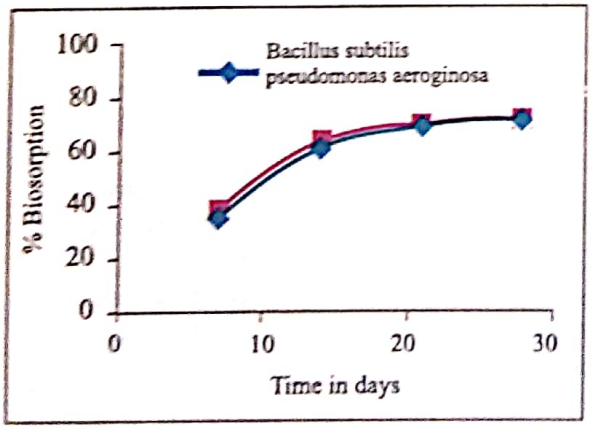

Figure 4 Effect of contact time on percentage manganese biosorption by *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Pseudomonas aeruginosa has a higher uptake of manganese than *Bacillus subtilis*. This may be due to Cell walls of gram negative bacteria being somewhat thinner than the gram positive ones and are also not heavily cross-linked. Eagon , (2004) and Vasanthy, (1984) explained the higher tolerance ability of the gram negative bacteria to heavy metals than the gram positive species. The maximum metal tolerance by the gram negative bacterial forms might be due to their abundant sedentary organism and also due to the metal precipitation in their peptidoglycan layers (Parameswari *et al.*, 2009). The lipopolysaccharides nature of the outer membrane of gram negative organisms is also responsible for efficient metal binding capacity (Mohanty *et al.*, 2004).

Conclusion

From this study both *Pseudomonas aeruginosa* and *Bacillus subtilis* were effective biosorbents in Manganese removal at varying conditions of pH, temperature, biomass concentration, contact time and initial metal concentration which are the major factors that affects biosorption processes. pH 5, temperature of 37°C, 2ml biomass concentrations, 28days contact time and 5ppm initial metal concentration were the optimum conditions found suitable for manganese biosorption. Although *Pseudomonas aeruginosa* and *Bacillus subtilis* were effective in Manganese Biosorption, *Pseudomonas aeruginosa* was found to be more effective in Manganese Biosorption hence a better alternative over *Bacillus subtilis*

References

Brenner, D. J., Krieg, N. R. and J. T. Staley (2005). *Bergey's Manual of Systematic Bacteriology*, volume 2 (Eds) Springer, New York.

Kanu, I. and O. K. Achi (2011). Industrial Effluents and Their Impact on Water Quality of Receiving Rivers in Nigeria, *Journal of Applied Technology and Environmental Sanitation*, **1(1)**: 75-86.

Ahalya, N., Ramachandra, T.V. and R. D. Kanamadi, (2003). Biosorption of Heavy Metals, *Research Journal of Chemistry and Environment* 7: 47-78.

Kratochvil, D. and B. Volesky (1998). Advances in the biosorption of heavy metals, *Trend in Biotechnology*, 16: 291–300.

Keen, C .L., Ensunsa, J. L. and M. H. Watson (1999). Nutritional aspects of manganese from experimental studies, *Neurotoxicology*. 20(2-3): 213-223.

Mohanty, S., Bapuji, M., Mishra, R. K., Sree, A., Ray, P., Mohapatra, S. B. and C. C. Rath (2004). Studies on metal tolerance of bacterial associates of marine sedentary organisms, *Asian Journal of Microbiology, Biotechnology and Environmental Science* 6(2): 291-296.

Eagon, G. R. **(1984).** *The resistance characteristics of Pseudomonas*, In development of Industrial microbiology

Parameswari, E., Lakshmanan, A. and T. Thilagavathi, (2009). Biosorption of Chromium (vi) and Nickel (ii) by bacterial isolates from an aqueous solution, *Electronic Journal of Environmental, Agricultural and Food Chemistry* 8 (3): 150-156.

Vasanthy, M. (2004). Biotechnology & Environmental Sciences, *Asian Journal of Microbiology, Biotechnology and Environmental Science* 6: 583-586.

Volesky, B. and Z. R. Holan (1995). Biosorption of heavy metals, *Biotechnology Prog.*11, 235–250.

Habitat, U.N. (2010). *The State of African Cities,* Governance Inequality and Urban Land Markets. UN-Habitat, Nairobi.

Vijayanand, S., Hemapriya, J., Selvin, J. and S. Kiran (2012). Operational Stability and Reusability of *Halobacterium sp.* JS Cells 1 Immobilized in Various Matrices for Haloalkalipuilic Protease Production, *International Journal of Microbiology Research,* 3(1): 1-6.

Volesky, B. and G. Naja (2005). *Biosorption: Application Strategies. International Biohydrometallurgy Symposium,* Cape Town, South Africa

Sharanagouda, U. and T. B. Karegoudar (2002). Degradation of 2- methylnaphthalene by free and immobilized cells of Pseudomonas sp. strain NGK1, *World Journal of Microbiology and Biotechnology,* 18: 225-230.

Adhinarayana, K., Jyothi, B. and P. Ellaiah (2005). Production of alkaline protease with immobilized cells of *Bacillus subtilisPE-11* in various matrices by entrapment technique, *AAPS Pharmaceutical Science and Technology,* 6: 91-97.

Ivánová, D., Horvathova, H., adukova, J. and J. Kavulicova (2010). Stability of immobilized biosorbents and its influence on biosorption of copper, *Nova Biotechnologica,* 10(1): 45-51.

Usha, M. S., Sanjay, M. K., Gaddad, S. M. and C. T. Shivannavar (2010). Degradation of H-Acid by Free and Immobilized cells of *Alcaligeneslatus. Brazilian Journal of Microbiology,* 41: 931-945.

Pehlivan. E., Tran, T.H., Ouédraogo, W.K., Schmidt, I. C., Zachmann, D. and M. Bahadir (2013). Removal of As(V) from aqueous solutions by iron coated rice husk, *Fuel Processing Technology.*106: 511-517.

Vijayaraghavan, K. Y. and S. Yun (2008). Bacterial biosorbents and Biosorption, *Biotechnology Advances,* 26; 266–291.

Vijayaraghavan, K., Joseph, R. J., Kandasamy, P., and V. Manickam (2004). Copper removal from aqueous solution by marine green alga Ulva reticulate. *Electronic Journal of Biotechnology* 7(1): 61-71.

Wang, J. and C. Chen (2009). Biosorbents for heavy metals removal and their future, *Biotechnology Advances* 27: 195–226.

Department of Health And Human Services (2012). Public Health Service (Manganese) Agency for Toxic Substances and Disease Registry.