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Biofilm and Trace Metals Removal from Waste Aqueous Effluent Using *Moringa oleifera*-Silver Nitrate Microparticles

Ukiwe L. Nnabugwu[1*] and Oki R. Remi[2]

[1]Department of Chemistry, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria.
[2]Department of Chemistry, Prairie View A&M University, P.O.Box 519, MS 2215 Prairie View, Houston, Texas, 77446, USA.

Authors' contributions

This work was carried out in collaboration between both authors. Authors ULN and ORR designed the study. Author ULN performed the statistical analysis and wrote the first draft of the manuscript. Author ORR wrote the protocol. Authors ULN and ORR managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The present research investigated the synthesis of a novel adsorbent by combining *Moringa oleifera* and silver nitrate ($AgNO_3$). The chemical components of the pre-adsorbent and adsorbent composites were characterized using Fourier Transform Infra-red spectroscopy (FTIR). The equilibrium adsorption capacity (Qe) for Cu, Mn and Fe in DMS_{H2O} adsorbent was 0.04, 0.04 and 0.04 mg/g, respectively, while that for Cu, Mn and Fe for DMS_{C2H5OH} adsorbent was also 0.04, 0.04 and 0.04 mg/g, respectively. The results indicate that the adsorbent was effective in removing Cu more than Mn and Fe from the aqueous salt mixture. Furthermore, the results observed for the biofilm experiment revealed that the *M. oleifera*-$AgNO_3$ biocidal action was effective in deactivating oxidizable components in the wastewater system after 8 days. T-test of difference=0, at df=12, P<.05 for metal adsorption and t-test of difference=0.04, at df=8, P<.05 for COD removal indicate no

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**Corresponding author: E-mail: luggil2002@yahoo.com;*

statistical significant difference in metals adsorbed and COD removed in the waste stream. The above findings have demonstrated that the synthesized *M. oleifera*-$AgNO_3$ adsorbent would be effective in cleaning-up waste streams contaminated with microbes and trace metals.

Keywords: Adsorption; contamination; environment; metals; plant; pollution; Moringa oleifera; wastewater.

1. INTRODUCTION

The need for high quality drinking water is increasing as non-polluted water sources are depleting due to effects of climate change and limited water sources. However, discharge criteria of wastewater are also becoming stricter since new legislations are coming on stream to prevent environmental pollution. Hence, water treatment has become very essential to meet the demand for portable water for consumption.

Trace metals are elements such as chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Zinc (Zn) etc that normally occur at very low levels in the environment. But in higher amounts, many of the trace elements have been linked to instances of chronic liver and kidney injury [1]. Concentrations of trace metals in the environment increase when they are released from rocks and industrial wastes. These releases can occur through natural processes or through human activities. Human activities that release trace metals into the environment include; mining, smelting, burning of coal, and wastewater disposal [1].

Moringa oleifera in the family of *Moringaceae,* is a popular shrub grown mainly in tropic climates. Variety of uses of the plant has been reported severally [2]. Most importantly, the plant has been used as a water treatment natural bioactive compound extract and coagulant for turbid water [3]. *Moringa oleifera* plant has been studied extensively for its biosorption characteristics. Biosorption is a physiochemical process that occurs naturally in certain biomass which allows the biomass to passively concentrate and bind contaminants onto its cellular structure [4]. Biosorption provides an economical alternative for removing toxic heavy metals from domestic and industrial effluent.

According to Adelaja et al. [5], the adsorption of lead (II) ions (Pb^{2+}) onto ground *M. oleifera* pods was observed to be dependent on the pH and concentration of the Pb^{2+} aqueous solution as well adsorbent dosage and contact time of the adsorption process. However, the study concluded that *M. oleifera* pods were not effective as biosorbent for removal of Pb^{2+} ions from wastewater at pH 7.0. In a related study, *M. oleifera* seeds were investigated as biosorbent for removing metal ions from domestic effluent [6]. The authors noted that the plant has an efficient morphological profile for adsorbing ions (Cd, Pb, Cu) from waste streams due to the presence of interstices and protein component resulting in a high heterogeneity of the seed's matrix.

Trace metals such as Cd, has been successfully determined in alcohol fuel using *M. oleifera* seeds as biosorbent [2]. The plant can also be used as a natural solid adsorbent for removing silver iodide (AgI) in aqueous solutions [7]. Recent advances have come to light in the application of *M. oleifera* plants as biosorbents in investigations conducted by Sharma et al. [3] and Sharma et al. [8]. Both studies observed that the *M. oleifera* plant could be applied in waste aqueous systems to effectively remove Cd as well as in ternary biosorption studies to remove Cd(II), Cr(III) and Ni(II), respectively. However, the use of *M. oleifera* in combination with silver nitrate as a micro-adsorbent has not been reported.

Biofilm consists of organic and inorganic materials, ranging from decaying products in the wastewater [9]. Biofilm formation occurs when microorganisms attach themselves to sub-surface structures in the water system. Biofilms develop quickly on iron than on plastic and polymer pipe surfaces. Iron pipes also encourage the composition of the microbial communities present in the biofilm [10]. In further studies, it was observed that biofilms in drinking water systems can serve as a reservoir for *Helicobacter pylori* (microorganism implicated in causing ulcers and cancer) [11]. Biofilms, while being a cause of concern for water utilities management, has been associated with the removal of some haloacetic acids (HAA) such as mono and dihalogenated species in drinking water distribution systems. HAA are commonly degraded by *Afipia felis* found in drinking water [12].

Several characteristics of the aqueous mixture such as pH, nutrients level, ionic strength, and temperature, have been noted to influence the rate of microbial attachment to a substratum, and hence increase biofilms proliferation [13]. Many researchers are of the opinion that a definite relationship exist between seasonal effects and biofilm formation in aqueous systems [14]. According to Fletcher [15], an increase in the concentration of Na^+, Ca^{2+}, and Fe^{3+} ions in aqueous systems influenced the attachment of *Pseudomonas fluorescens* on glass surfaces; apparently, the ions reduced the repulsive forces between the negatively charged bacterial cells and the glass surfaces.

Silver (Ag) and silver ions (Ag^+) have been reported to have strong bacterial inhibition. Its biocidal effects when applied to pathogenic bacteria as disinfectant agents, is not in doubt [16,17].

The antimicrobial effect of Ag and Ag^+ on different types of microorganisms has been investigated by several researchers [16,17]. To render the cell inactive, Ag and Ag^+ binds to disulphide or sulfhydryl groups in the cell wall proteins and also to the deoxyribonucleic acid molecule in the cell. These binding actions disrupt metabolic processes in the cell and lead to cell death [18,19].

Silver has been applied as disinfectant in water filters against planktonic bacteria [20]. In combination with copper, Ag has been used effectively in water distribution systems against *Legionella pneumophilia* [21,22].

Silver and Ag nanoparticles (AgNPs) have general water disinfection anti-bacterial properties against a wide range of gram-negative and gram-positive bacteria, which include; *Escherichia, Salmonella, Clostridium* and *Staphylococcus* [23]. However, recent findings by Sun et al. [24] and Elechiquerra et al. [25] have suggested that AgNPs also has biocidal action against fungi such as *Aspergillus niger, Candida albicans* and viruses such as hepatitis B and the human immune deficiency virus (HIV-1). There are indications that high doses or repeated administration of Ag and Ag^+ ions (approximately 10 g) can result in signs of mild poisoning considered to be of no toxicological significance [26].

Therefore, *Moringa oleifera*-$AgNO_3$ synergy, is an innovative combination when applied to treat water and wastewater.

2. MATERIALS AND METHODS

2.1 Adsorbent Preparation

Approximately 100 ml of a 0.1 mg/l silver nitrate ($AgNO_3$) solution was measured into a 500 ml beaker, and 100 g of *Moringa oleifera* seeds, whose external kernel shells have been removed and the kernels previously washed and rinsed with deionized water were added to the $AgNO_3$ solution in the 500 ml beaker. About 10 ml solution of a 25% $^v/_v$ tetramethylsilane in ether was also measured and added to the *M. oleifera*-$AgNO_3$ mixture. The mixture was allowed to stand for 48 h to allow the seeds adsorb the aqueous mixture. The *M. oleifera* seeds were recovered after this period and sun-dried for another 48 h and further placed in an oven (AEG 60 cm, Canberra, Australia) at 70ºC until constant weight.

Adsorbents of the *M. oleifera* seed were prepared by grinding the seeds manually to 5 mm. The ground seeds were divided into two parts. One part was washed in 10 ml of deionized H_2O, while the other part was washed in a 10% $^v/_v$ solution of alcohol. Both washed seeds were dried in an oven at 50ºC for 1 h. Washing with these solvents introduces hydroxyl groups in the adsorbent, restrict particle agglomeration and increase adsorption capacity. The dried *M. oleifera* seeds were further labelled DMS_{H2O} and DMS_{C2H5OH}, respectively. About 1 g each of both DMS_{H2O} and DMS_{C2H5OH} were recovered to obtain the Infra-red spectra (Shidmazu FTIR, 8400S, Japan) of the pre-adsorbent materials. Adsorbents of DMS_{H2O} and DMS_{C2H5OH} were prepared by placing the entire quantity of both washed materials in a crucible and heat-treated in a furnace (Mini FurnaceTZ-1200C, Shanghai, China) at 200ºC for 1 h. The adsorbents were recovered from the furnace and cooled in a dessicator. About 1 g of the synthesized adsorbent each from DMS_{H2O} and DMS_{C2H5OH} were also withdrawn and used to obtain infra-red spectra of the synthesized adsorbent materials.

2.2 Equilibrium Adsorption Capacity Measurement (Qe)

Approximately 90 ml mixture consisting of a 30 ml solution of a 1 g/l each of the following salts $FeSO_4$, $CuSO_4$ and $MnSO_4$ was measured into a 200 ml beaker. The pH of the mixture was recorded using a pH meter (PHS 25, Shanghai Automation Instr. Corp., China) and adjusted to pH=4 using 20% ($^w/_v$) NaOH and 0.1 M HCl, added as appropriate. Exactly, 2 g of the synthesized DMS_{H2O} was weighed and added to the mixture in the 200 ml beaker. The adsorbent-salt solution mixture was stirred for 5 min and allowed to stand for 20 min. After this period about 5 ml of the mixture was filtered (Whatman No 42 filter paper) and used to determine the concentration of the metals (Fe, Cu, and Mn) in the mixture using a Multi-parameter photometer (H183200, HANNA Instr. UK). Three repetitions were made and the mean adsorption capacity (Q) of the adsorbent was estimated using the following equation:

$$Q = \frac{(Co - Ce)(V)}{M} \quad (1)$$

Where

Co= Initial concentration of the salt used
Ce= Concentration of the residual metal in the salt mixture after sorption
V = Volume of prepared salt mixture
M = Mass of adsorbent

The concentration of the residual metals in the salt mixture was determined intermittently at 10 min interval. A graph of Q versus time (t) was plotted, and the constant value of Q was referred as the equilibrium adsorption capacity (Qe).

This procedure was repeated for the synthesized DMS_{C2H5OH}.

2.3 Biofilm Experiment

The wastewater used for this research was obtained from a waste pipe channelling wastewater from the students Hostel A residence of the Federal University of Technology, Owerri, Nigeria.

A 1L plastic bottle was used to collect the wastewater and this was transported to the Project Laboratory of the Chemistry Department, Federal University of Technology, Owerri, Nigeria. About 100 ml each of the wastewater was poured into two separate 200 ml beakers, previously, washed and rinsed with distilled water and labelled BE 1 and BE 2.

About 10 ml of the wastewater in BE 1 was measured into a 50 ml conical flask. The pH of the wastewater was recorded using a pH meter (PHS 25, Shanghai Automation Instr. Corp., China) and adjusted to pH=8 using 2 ml of a 20% ($^w/_v$) NaOH, added as appropriate. Exactly, 3 ml of a 0.01 M potassium dichromate reagent and 7 ml of a10 M sulphuric acid reagent were separately measured and poured into the 50 ml conical flask.

The top of the flask was capped using a rubber cork. The mixture was stirred for 3 min and placed inside a chemical oxygen demand (COD) digester (2015D, Spectralab Intr. Ltd, India) at 110 ^oC for 30 min. After this period, the conical flask was withdrawn from the digester and cooled in a water bath (WBH14, GENLAB, UK) to room temperature. About 3 drops of ferroin indicator were added to the digestate in the conical flask and the content titrated against a 0.1 M ferrous ammonium sulphate (FAS) solution to a reddish brown color. Three repetitions were performed and the mean COD (mg/l) concentration was obtained. A control sample was processed as in above without adding the wastewater sample. The mean COD removed in the mixture was obtained using the following equation;

$$COD = (X - Y) \times M \times 8000/ml \quad (2)$$

Where

X = ml of FAS solution for the control experiment
Y = ml of FAS solution for the sample
M = Molarity of FAS
8000 = Milli equivalent weight of oxygen x 1000 ml/l

Exactly, 2 g of the synthesized adsorbent DMS_{H2O} was added to BE 2. The mixture was stirred continuous for 10 min and the COD of the mixture was estimated after two days as described above for BE 1. This procedure was repeated for 10 days at interval of 2 days.

However, the procedure was also repeated using the synthesized DMS_{C2H5OH} adsorbent.

2.4 Statistical Analysis

Arithmetic mean and standard deviation were used to present data. The percentage relative error was used to estimate how closely measured metal concentration relates, while the t-test was also used to estimate significant difference in adsorbed trace metals concentrations between the DMS_{H2O} and DMS_{C2H5OH} synthesized adsorbents and concentrations of COD (mg/l) of wastewater at various times for synthesized adsorbent DMS_{H2O}

and DMS_{C2H5OH}. Data were analysed using Microsoft 2000 Excel (Version 9).

3. RESULTS AND DISCUSSION

Table 1 show the Infra-red spectra peak characteristics for DMS_{H2O} and DMS_{C2H5OH} pre-adsorbent and synthesized adsorbent composites, respectively. The peaks around 3944 and 3813 cm^{-1} represent medium and strong bonded OH groups, while that at 1750 cm^{-1} is indicative of unsaturated ketone group for DMS_{H2O} pre-adsorbent composite. A slight shift of these peaks was observed around 3940-3755 cm^{-1} and 1748 cm^{-1} for the synthesized adsorbent composite. Nonetheless, the pre-adsorbent substance of DMS_{C2H5OH} showed peaks at 3906, 3817 and 1735, cm^{-1}, also representing medium and strong bonded OH groups and ketones. A slight shift of these peaks was also observed around 3975, 3770 and 1744 cm^{-1} for the synthesized DMS_{C2H5OH} material.

The peaks observed at 3334 cm^{-1} and 3381 cm^{-1} represents adsorption peaks for DMS_{H2O} and DMS_{C2H5OH} pre-adsorbent composites, respectively, while the peaks at 3357 cm^{-1} and 3384 cm^{-1} also represents adsorption peaks for DMS_{H2O} and DMS_{C2H5OH} synthesized adsorbent materials, respectively. These adsorption peaks were assigned to N-H medium stretching primary amine. The presence of such functionality in the structure of the *M. oleifera* plant has been reported [27]. We can conclude that the shifts in peak values observed could be as a result of formation of chemical bond between functional groups and Ag^+ ions during calcining. This line of reasoning is consistent with findings published by Bello et al. [28], Al-Ghouti et al. [29] and Bekci et al. [30].

The absorption peaks around 486-698 cm^{-1} and 528-613 cm^{-1} represents adsorption peaks for DMS_{H2O} and DMS_{C2H5OH} synthesized adsorbent materials, respectively. These peaks are assigned to $C-Ag^+$ ions bond formed during calcining. Formation of such sharp absorption $C-Ag^+$ ion bond has been reported Sheibley et al. [31].

Table 2 shows concentrations (g/l) of residual metals in salt mixture at various times for synthesized adsorbent DMS_{H2O} and DMS_{C2H5OH}. Table 3 shows the effect of time (min) on the adsorption capacity (Q) of the synthesized adsorbent DMS_{H2O} and DMS_{C2H5OH}, respectively. The values obtained for the residual metals in the salt mixture raise an important issue with respect to pH dependence by adsorbents for metal adsorption. Given the values observed for the adsorption capacity in the present study, we conclude that the ability of the adsorbents to preferentially adsorbed metals may have been pH dependent. This finding is in agreement with recently published data by Salehzadeh (32), who observed that adsorption capacity of heavy metals by *Xanthium pensylvanicum* was pH dependent. It was observed that the equilibrium adsorption capacity (Qe) for Cu, Mn and Fe in DMS_{H2O} adsorbent was 0.04, 0.04 and 0.04 mg/g, respectively, while that for Cu, Mn and Fe for DMS_{C2H5OH} adsorbent was 0.04, 0.04 and 0.04 mg/g, respectively. These results indicate that the adsorbent was able to remove, approximately, the same concentrations of Cu, Mn and Fe from the aqueous salt mixture and, hence, the Qe values of the three metals did not show much difference between their removal ratios. However, the results obtained also indicated that washing the *M. oleifera*-$AgNO_3$ substance with water or ethanol had no effect on the adsorption capacity of the synthesized adsorbent.

Table 4 shows values of concentrations of COD at various days for DMS_{H2O} and DMS_{C2H5OH} adsorbents, respectively. For DMS_{H2O}, the COD of the wastewater increased appreciably from day 1 to 4. The reason for this phenomenon could be due to the increase in biofilms growth as a result of the warm environment (increased temperature) in the laboratory and within the reacting system. Such growth condition of microbes has been reported [33]. However, it was observed that after day 4 the COD of the wastewater started decreasing due to the impact of the *M. oleifera*-$AgNO_3$ biocidal action.

It was observed for the DMS_{C2H5OH} adsorbent that the COD marginally increased from day 1 to day 2, due to reasons described as in above, but steadily decreased from day 4. As from day 8, the COD was zero (0) indicating that there was no bacteria activity in the experimental system, hence, no demand for oxygen, since no oxidizable components were present in the wastewater.

The percentage relative error for Cu, Mn and Fe for DMS_{H2O} and DMS_{C2H5OH} synthesized adsorbents are 3, 2 and 0.6% (DMS_{H2O}), and 0.7, 0.0 and 8% (DMS_{C2H5OH}), respectively. Values for Cu adsorption using DMS_{H2O} and DMS_{C2H5OH} adsorbents were used to test for significant difference in metal adsorption by the synthesized adsorbents. T-test of difference=0, at df=12, P<.05 for metal adsorption and t-test of difference=0.04, at df=8, P<.05 for COD removal indicate no statistical significant difference in metals adsorbed and COD removed in the waste stream.

Table 1. Infra-red spectra peak characteristics of DMS_{H2O} and DMS_{C2H5OH} pre-adsorbent and synthesized adsorbent composite

Bond	Wavelength (cm^{-1})			
	DMS_{H2O}		DMS_{C2H5OH}	
	Pre-adsorbent	**Adsorbent**	**Pre-adsorbent**	**Adsorbent**
O-H medium-strong bonded stretching	3813-3944	3755-3940	3817-3906	3770-3975
N-H medium stretching	3334	3357	3381	3384
C=O strong stretching	1750	1748	1735	1744
$C\text{-}Ag^{+}$		486-698		528-613

Table 2. Concentrations (g/l) of residual metals in salt mixture at various times for synthesized adsorbent DMS_{H2O} and DMS_{C2H5OH}

Adsorbent	DMS_{H2O}			DMS_{C2H5OH}		
	Cu	**Mn**	**Fe**	**Cu**	**Mn**	**Fe**
Time (min)	**Mean (g/l)**	**± SD**		**Mean (g/l)**	**± SD**	
20	0.08 ± 0.1	0.10 ± 0.1	0.07 ± 0.2	0.05 ± 0.0	0.10 ± 0.0	0.03 ± 0.0
30	0.01 ± 0.2	0.10 ± 0.1	0.05 ± 0.1	0.06 ± 0.1	0.01 ± 0.1	0.03 ± 0.0
40	0.04 ± 0.1	0.08 ± 0.1	0.05 ± 0.0	0.07 ± 0.1	0.01 ± 0.2	0.06 ± 0.1
50	0.04 ± 0.1	0.08 ± 0.1	0.05 ± 0.0	0.07 ± 0.0	0.01 ± 0.1	0.05 ± 0.0
60	0.06 ± 0.0	0.05 ± 0.1	0.04 ± 0.1	0.07 ± 0.0	0.01 ± 0.1	0.06 ± 0.1
70	0.06 ± 0.1	0.10 ± 0.1	0.05 ± 0.1	0.07 ± 0.0	0.01 ± 0.0	0.06 ± 0.2
80	0.04 ± 0.0	0.02 ± 0.0	0.04 ± 0.1	0.06 ± 0.1	0.01 ± 0.0	0.05 ± 0.0

Table 3. Values of adsorption capacity (Q) at various times for synthesized adsorbent DMS_{H2O} and DMS_{C2H5OH}

Adsorbent	DMS_{H2O}			DMS_{C2H5OH}		
	Cu	**Mn**	**Fe**	**Cu**	**Mn**	**Fe**
Time (min)	**Mean(mg/g)**	**± SD**		**Mean (mg/g)**	**± SD**	
20	41.4 ± 0.1	44.5 ± 0.1	41.8 ± 0.1	42.7 ± 0.1	40.5 ± 0.1	43.6 ± 0.2
30	44.5 ± 0.1	44.5 ± 0.2	42.7 ± 0.1	42.3 ± 0.1	44.5 ± 0.1	43.6 ± 0.2
40	43.2 ± 0.1	41.4 ± 0.1	42.7 ± 0.1	41.8 ± 0.1	44.5 ± 0.1	42.3 ± 0.1
50	43.2 ± 0.1	41.4 ± 0.1	42.7 ± 0.1	41.8 ± 0.1	44.5 ± 0.1	43.7 ± 0.1
60	42.3 ± 0.1	42.7 ± 0.0	43.2 ± 0.1	41.8 ± 0.1	44.5 ± 0.1	42.3 ± 0.1
70	42.3 ± 0.1	40.5 ± 0.0	42.7 ± 0.1	41.8 ± 0.1	44.5 ± 0.2	42.3 ± 0.1
80	43.2 ± 0.2	44.1 ± 0.1	43.2 ± 0.2	42.3 ± 0.1	44.5 ± 0.1	42.7 ± 0.1

Table 4. Concentrations of COD (mg/l) of wastewater at various times for synthesized adsorbent DMS_{H2O} and DMS_{C2H5OH}

Adsorbent	DMS_{H2O}	DMS_{C2H5OH}
	COD	**COD**
Days	**Mean (mg/l) ± SD**	**Mean (mg/l) ± SD**
2	224 ± 0.1	232 ± 0.2
4	1040 ± 0.1	80 ± 0.2
6	640 ± 0.2	60 ± 0.1
8	80 ± 0.1	0 ± 0.0
10	26 ± 0.1	0 ± 0.0

**COD value of raw wastewater for day 1 = 173 mg/l*

4. CONCLUSION

The present research had synthesized a novel adsorbent using *M. oleifera*-$AgNO_3$, for removing trace metals (Cu, Mn and Fe) from the aqueous salt mixtures as well as preventing proliferation of biofilms (microbes) in wastewater. The results obtained have shown that *M. oleifera* seeds are effective as composites adsorbent for removing metals from aqueous systems. The shift in the FTIR spectra obtained for the pre-adsorbent and adsorbent composites, and the values obtained for the equilibrium adsorption capacities (Qe) as well as COD, have demonstrated that the *M. oleifera*-$AgNO_3$ adsorbent was not just a simple mixture of both substances but that a synergic interaction existed between the *M. oleifera* and $AgNO_3$ compound which should have occurred during the synthesis of the composites. However, it was also observed in the present study that washing the synthesized adsorbent with either H_2O or C_2H_5OH didn't influence the adsorption capacity of either adsorbent.

Mn, Fe, and Cu were the elements of interest in the present study because, although not a danger to human health when present in water at concentrations within recommended guideline values, intentionally high presence or high intake of Mn, Fe and Cu may cause severe problems which include; biofouling in distribution networks, staining of laundry, nausea, liver and kidney damage, and even death [34,35].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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