

Antimicrobial Activity of Silver-Zeolite LTA on Heavily-Contaminated Underground Ghanaian Waters

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Abstract

Zeolite LTA was synthesized in the laboratory and loaded with silver ions by isomorphous substitution technique. The silver-loaded zeolite A (Ag-LTA) was characterized by X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and Fourier transformed infrared (FTIR) spectroscopy. XRD and SEM analysis showed little or no changes in the phase purity of all zeolites before and after ion exchange or before and after substitution of silver ions. The antimicrobial activity of the silver-loaded zeolite A was investigated by exposing well water samples from Vui community in the Volta Region of Ghana. Parameters such pH, conductivity and temperature as well as microbial analysis were measured before and after addition of silver-

Keywords: Synthesis, zeolite LTA, underground water, silver-zeolite.



A. INTRODUCTION

Water, the most abundant raw material on the Earth's surface like any other raw material must be treated before consumption due its universal solvency. Water treatment in general is the various processes carried out on water obtained from natural sources to render it fit for human consumption. According to Tebbutt [26], Montgomery [28] and Rangwala [27], water meant for human consumption must be free from all harmful impurities. Well water including spring waters (ground and surface water) are water that come from deep in the ground through mineral layers [17]. Hence, well waters contain inorganic salts such as calcium and magnesium as well as organic matter. Ground water abstractions in Ghana have been reported to be over 56000 comprising boreholes, hand-dug wells and dugouts [16]. Although perceived to be of more stable and better microbial quality the physical and microbial quality of Ghanaian well water is doubtful [24]. Furthermore, a number of rural folks where water treatment is either absent or very poor have been reported to suffer from water related diseases such as diarrhea, typhoid, cholera and hepatitis A [15, 18]. The cause of such diseases are attributed to anthropogenic activities such as nearness pit latrines, cemeteries, refuse dumps and septic tanks, to wells and boreholes as well as poor hygienic conditions. There has been extensive research into the use of antimicrobial agents. Among the antimicrobial agents silver has been reported to be the most effective agent due to its ability to affect many different functions of microbial cells and non-selective in its

antimicrobial action. Hence, it has antimicrobial activity against a broad spectrum of microorganisms. However, silver ions in any normal containing agent leaches out quickly thereby producing a short resident time.

Zeolites have been found to be an attractive reservoir for silver ions. Zeolites are crystalline hydrated aluminosilicates whose framework structure consists of cavities or pores that are occupied by cations or water molecules. Both the cation and the water molecule have considerable freedom of movements and this permits ion exchange and reversible dehydration [20, 23]. The zeolite structure is made up of a central atom commonly silicon or aluminum surrounded by four oxygen atoms. The tetrahedral atoms, called T-atoms are stacked in beautiful regular three-dimensional arrays such that channels. The zeolite frameworks are typically anionic which are counterbalanced by the positive charges of cations resulting in a high cation exchange capability [19, 21]. Exchangeable cations include lithium, cadmium, lead, zinc, copper, ammonia, silver and protons. The zeolite channels or pores are microscopically small, and have molecular size dimensions in such a way that they are normally termed molecular sieves [23]. In this work, silver ions were incorporated into zeolite type LTA in order to release the silver ions in a controlled and sustained manner. The antimicrobial activity of the silver-zeolite LTA (Ag-LTA) produced was investigated by exposing samples of well and borehole waters from Vui in the Keta District of the Volta Region of Ghana and the extent of removal of total fecal coliforms were determined.

B. METHOD

Water samples were collected from eleven wells in Vui community: eight from the town and three from around the Vui cemetery, two boreholes also in Vui town and one from the tap. The eight samples from the township were labeled K1, K2, K6, K7, K8, K9, K10 and K11. The three samples taken from the cemetery were labeled K3, K4 and K5. In addition, the samples taken from the boreholes were labeled K8B and K9B and the tap water sample was labeled K12. Duplicate samples were collected from the wells and boreholes in sterilized 1.5 L plastic and glass bottles and kept in a container packed with dried ice cubes. The samples were analyzed within 48 hours after collection. Physical parameters such pH and conductivity were measured for each sample. Silver-zeolite LTA was produced in the Water Research laboratory, Department of Civil Engineering, KNUST and characterized in the University of Wolverhampton, UK. One-quarter strength Ringer solutions were prepared by dissolving 1 tablet in 500 ml de-ionized water in a 1 L flask. MacConkey broth was prepared following the manufacturer's protocol. All media were sterilized by autoclaving at 121 °C for 15 minutes. 0.723 g of sodium hydroxide (Aldrich Chemicals, UK) was dissolved in 80 ml of distilled water. The solution was divided into two equal halves and each transferred into plastic beakers. 0.258 g of sodium aluminate (Fischer Scientific Chemicals, UK) was added to the first half with continuous stirring until a homogeneous solution was obtained. 15.48 g of sodium metasilicate (Fischer Scientific Chemicals, UK) was added to other half while mixing until the gel was homogenized. The two samples were mixed quickly and the mixture was again stirred continuously until a homogeneous solution was obtained. 5 ml aliquot of a 0.5 M silver nitrate

solution was added to the gel and thoroughly stirred in the dark until a homogeneous phase was obtained. The gel was poured into PTFE-vessels, with each vessel containing about 10 g of the gel. The bottles were put into an electric oven at a temperature of 99 °C for 4 hours. The reaction in the PTFE bottles was quenched by running cold water on the bottles after they were removed from the oven until they were cooled to room temperature. The synthesized samples were filtered using a Buchner vacuum funnel and Whatman No 1 filter paper. The powder samples obtained were washed copiously with 500 ml of distilled water. Following overnight drying of the powdered zeolite at 40 °C in an electrical oven, the zeolite was crushed into uniform powder with pestle and mortar, sieved and stored. As the silver zeolite LTA used for this study was produced in our laboratory, it's essential to characterized it according to its structure, phase purity, chemical composition, molecular vibration states, thermal stability and surface morphology. The phase purity of the Ag-LTA particles was analyzed with X-ray diffraction.

The surface morphology and size of the Ag-LTA was examined by scanning electron microscopy (SEM). The chemical composition of Ag-LTA was determined by energy dispersive X-ray analysis (EDX). Thermo Gravimetric Analysis was used to determine the thermal stability of the Ag-LTA and Fourier transform infrared was used to determine the vibrational properties. To enumerate the *E. coli*, total and fecal coliforms 1 ml of each of the samples was serially diluted up to 10⁻³ in a sterile Ringer solution. Starting with sample K1, 1 ml aliquot of each of the dilution were added aseptically to MacConkey broth after which Durham tubes were inverted in the MacConkey broth. Following incubation at 37 °C for 48 hours colour changes in each of the tubes were observed. Tubes with change in colour from pink to yellow indicated the presence of total coliforms and were recorded as positive whereas tubes with no change in colour were recorded as negative. Fecal coliforms were enumerated by preparing a sterilized and unsterilized inoculum cultures. A sterile inoculating loop was inserted into the sterilized bottle containing sterile inoculums and aseptically transferred into another sterilized bottle containing MacConkey broth. The same procedure was repeated for the unsterilized inoculum cultures. The bottles were incubated at 37 °C for 24 hours. After which they were removed, gently shaken to observe the production of gas. The whole procedure was repeated for all the other water samples. Bottles in which there was gas production were recorded as positive and those with no gas production as negative. For the enumeration of *E. coli*, a single total coliform-positive colony was aseptically inoculated into an EC-MUG contained in a sterile test tube. The inoculated tubes were gently shaken to insure adequate mixing after which they were incubated in a water bath at 44 °C for 2 hours. Following incubation, the samples were taken to a dark room to observe whether or not fluorescence with The biocidal effect of Ag-LTA as a function of time was investigated by the method given by Kwakye-Awuah et al. with some modifications. A 1000 ml measuring cylinder was used to measure 200 ml of each sample into sterilized conical flasks labeled K1, K2, K3, K4, K5, K6, K7, K8, K9, K10 and K11. Starting with sample K1, 10 mg portion of the Ag-LTA was weighed using an electronic balance and added to the water sample after which the flask was covered with a foam. The procedure was repeated for the rest of the samples. Aliquot portions were withdrawn and analyzed for enumeration of total and fecal coliforms.

The flasks containing the Ag-LTA were placed in a rotary shaker and aliquots were withdrawn at 10, 20, 40, and 60 minutes. The enumerated for each sampling time.

C. RESULTS AND DISCUSSION

SEM micrograph of the synthesized zeolite LTA and silver-zeolite LTA by isomorphous substitution of silver ions is shown in Figure 1. The crystal morphology showed cubic arrangement in both synthesis routes. Hence, the isomorphous substitution did not alter the crystal properties of the zeolite crystals as confirmed by the XRD spectra (Figure 2). The XRD spectra also showed that the zeolite particles were highly crystalline with no impurity phases present. 93.35 showing that Ag-LTA was highly crystalline and that the proportion of absorbed water is very small. A marginal weight loss of 0.2 % of the total weight of Ag-LTA occurred at 450 °C and can be attributed to loss of chemically bound and adsorbed water within the zeolite framework. The mid-FTIR spectra of the as-synthesized zeolite A (red line) and silver-exchanged zeolite A (blue line) are given in Figure 4 in the region of lattice vibrations (1200 – 400 cm⁻¹). A large broad band was observed in the 977 cm⁻¹ in both samples. This band can be attributed to the overlap of the asymmetric vibrations of Si – O (bridging) and Si – O- (non-bridging) bonds. The band shifted towards a slightly higher frequency of 978 cm⁻¹. EDX spectrum detected silver ions in the zeolite framework after loading (Figure 5). The elemental composition obtained (Table 1) indicates that the atomic concentration of sodium ions decreased by 43.4 % after ion exchange. Aluminium ions decreased by 3.9 %. The atomic concentration of silver ions on the other hand increased from near zero to 6.8 % whilst that of silicon remained fairly constant after ion exchange. Thus the ion exchange of sodium ions by silver ions is likely to have been at the exchangeable sites within zeolite X framework. Results from the microbial analysis before Ag-LTA addition showed the presence of microbial indicator organisms in the samples obtained from the all the eight wells in the Vui community. In these wells the most probable number of total.

E. coli were also enumerated to be between and MPN /100 ml. For samples K9, K10 and K11 obtained from wells from the Keta government cemetery, only K9 contained MPN/100 ml of total coliforms, MPN/100 ml of fecal coliforms and MPN/100 ml *E. coli*. The rest of the samples showed negative results which meant they were somehow devoid of microorganisms. Fecal coliforms of and MPN/100 ml were recorded for samples KB8 and KB9 obtained from boreholes from the Vui community. According to WHO guidelines for potable water there should be complete absence of microbial indicator organisms in any 100 ml of sample of drinking water and not more than 50 total coliforms per 100 ml sample [24, 25, 30 – 32]. It is clear from Tables 4 – 7 that most the wells and boreholes did not meet this required guideline. The existence of total coliforms in water samples has been found to be indication of the presence of pathogenic bacteria such as *Klebsiella*, *Enterobacter*, *Salmonella* spp, *Shigella* spp, *V. cholera*, *Campylobacter*, *Jejuni*, *Campylobacter coli*, and pathogenic *E. coli* [33]. Such microorganisms could cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever among others [3, 4]. Furthermore, Cairncross [1], Kumar, [2] and Jung et al [14] reported that infectious dosage of most of these bacteria could range from whereas others

like enteric bacteria (gram-negative) could be as low as . The results of this study showed that the use of the wells and boreholes in the Vui community for drinking purposes is likely to cause serious health implications to consumers without prior treatment. . Upon the addition of Ag-LTA no indicator microorganisms were observed and enumerated after 10 minutes (Table 5), 20 minutes (Table 6) and after 30 minutes (Table 7). Silver ions have been shown to exhibit both biocidal and bactericidal activity [11, 13, 29]. Although the mechanism of antimicrobial action of silver is not clearly understood to date, its antimicrobial activity is known to affect microorganisms through a number of pathways. The ionic species carries a strong positive charge so it has high affinity for negatively charged groups of biological molecules [5]. These molecules include groups such as sulfhydryl, carboxyl, phosphates and other groups commonly found on macromolecular structures distributed throughout microbial cells [6 – 8]. The binding reaction alters the molecular structure of the macromolecule, rendering it worthless to the cell. The attack effectively inactivates many functions such as cell wall synthesis, membrane transport, nucleic acids such as RNA and DNA synthesis and translation, and protein folding and function [9, 10]. Because silver affects so many different functions of microbial cells, it is non-selective, resulting in antimicrobial activity against a broad spectrum of microorganisms including bacteria, fungi, and yeasts.

D. CONCLUSION

The study was conducted to assess the microbial parameter of samples of well and borehole water from Vui community in the Keta District, Volta Region, Ghana and remove the microbial contamination by the action of silver ions incorporated into zeolite LTA framework. Silver-doped zeolite LTA was successfully synthesized in the laboratory as evident in the SEM, XRD, TGA and FTIR results. The synthesized zeolite LTA was applied to heavily-polluted underground waters in Ghana. The microbial parameter of the samples was quantitatively determined and the antimicrobial activity of silver zeolite LTA was shown to be effective, the samples being completely devoid of microorganisms. Since zeolites are inert there as no interaction of the zeolite and microorganisms. The zeolite therefore loosely held the silver ions and released them in a controlled and sustained manner. Future work will explore the longevity and efficiency of antimicrobial action against broad range of microorganisms. Further work will examine the mode of antimicrobial activity on different strains. From the results obtained silver-zeolite LTA has attractive features which make it potentially useful in fabricating operating theater furnitures and fittings.

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