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Removal of *Escherichia coli* and heavy metals from aqueous solutions using silver-modified clinoptilolite

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ABSTRACT

The aim of this study was to investigate the removal of *Escherichia coli* and heavy metals (Pb^{2+}, Cd^{2+} and Zn^{2+}) from aqueous solutions using silver-modified clinoptilolite through the combined disinfection of *E. coli* by the silver ions and sorption of heavy metals on clinoptilolite. The silver-modified zeolites exhibited excellent disinfection performance with 100% removal of *E. coli* within 30 min. The as-received natural zeolites showed no disinfection characteristics under the same conditions. High metal removal efficiencies up to 92% were achieved with respect to the metals present in solution. In the *E. coli*-metals solution systems, disinfection was enhanced by the presence of metal ions which resulted in a relatively reduced amount of metals adsorbed by the zeolites due to the uptake of metal ions by the bacterial cells present the solution. The results of this study demonstrate that silver-modified clinoptilolite has a potential for the simultaneous removal of metals and pathogenic organisms from contaminated aqueous streams.

Keywords: Silver-modified clinoptilolite; Disinfection; Adsorption; *Escherichia coli*; Heavy metals

1. Introduction

Wastewater contains harmful pollutants such as pathogenic micro-organisms which cause waterborne diseases and heavy metals from industrial activities which are persistent non-biodegradable pollutants with adverse long-term effects on human health. Due to rapid population growth and increased industrial activities, large volumes of wastewater are being generated requiring more treatment technologies that can handle multiple pollutants [1–5].

Natural zeolites such as clinoptilolite are crystalline porous aluminosilicate minerals with exchangeable cations which balance the original charge of the zeolite framework. The characteristics of these zeolites make them ideal for use in adsorption, ion exchange and catalysis [4,6]. The use of natural zeolites in wastewater treatment has been extensively investigated and documented due to their high exchange capacities, cost effectiveness, environmental compatibility and high selectivity towards metal ions, with high metal removal efficiencies and adsorption capacities achieved in several studies [1–5,7–9].

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The disinfection applications of antibacterial silver ions and nanoparticles incorporated into different inorganic support materials from which they are slowly released have been demonstrated and reported in the literature [10–15]. Studies have shown that silver-modified clinoptilolite effectively eliminates micro-organisms from wastewater [10–12]. However, the emphasis has been on the antibacterial activity of the silver-modified zeolites and their performance in handling bacterial contamination.

Hence, the objective of this study was to investigate the dual treatment capacity of silver-modified zeolite–clinoptilolite for the removal of *Escherichia coli* as an indicator organism and heavy metals (Pb^{2+}, Cd^{2+} and Zn^{2+}) from aqueous solutions through the combined disinfection of *E. coli* by the silver ions and sorption of heavy metals onto clinoptilolite.

2. Materials and methods

2.1. Materials, micro-organism and reagents

Natural clinoptilolite as-received (particle size 0.2–0.6 mm) was washed with deionized water to remove fine particles and dried at 60˚C. *E. coli* NCIMB 8,545 used as an indicator of faecal contamination was grown in nutrient broth at 37˚C for 18 h and the cells were centrifuged, washed and suspended in deionized water. The following analytical grade reagents purchased from Fisher Scientific, UK were used to prepare metal stock solutions in deionized water: $AgNO_3$ (CAS No.: 7761-88-8); Pb $(NO_3)_2$ (CAS No.: 10099-74-8) $CdN_2O_64H_2O$ (CAS No.: 10022-68-1) and Zn $(NO_3)_2.6H_2O$ (CAS No.: 10196-18-6).

2.2. Silver modification of clinoptilolite

50 g of natural clinoptilolite (NZ) was suspended in 100 ml 3% (w/v) $AgNO_3$ solution at pH 5 and agitated for 24 h in the dark due to the light sensitivity of silver. The zeolites were then separated by filtration, washed and dried at 60˚C. The amount of silver taken up by the zeolite was 47.72 mgAg/g. Characterization of the natural (NZ) and silver-modified (AgZ) zeolites was carried out using a Hitachi S3200 N Scanning Electron Microscope coupled with an Energy Dispersive X-ray detector (SEM-EDX).

2.3. Batch disinfection experiments

Experiments were carried out using both AgZ and NZ. 0.2 g of zeolites was added to 50 ml *E. coli* (106–108 CFU/ml) solutions at pH 5 to maintain the same conditions as in the metal and *E. coli*-metal solutions in which metal precipitation had to be avoided. Control solutions without any zeolites added were also tested. The samples were shaken for 0–60 min at room temperature and enumeration of viable bacteria was done using the membrane filtration method [16]. The amount of silver released into the treated solutions was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

2.4. Batch metal adsorption experiments

A sample of 0.2 g of zeolites was suspended in 50 ml metal solutions (0.5 mg/l) at pH 5 to prevent precipitation and the solutions including controls (containing no zeolites) were shaken for 60 min at room temperature. Preliminary experiments showed that the optimal pH for metals removal was 5 and 60 min of contact was sufficient to reach equilibrium. The treated solutions were filtered using a 0.45 μm syringe filter, acidified to pH < 2 and refrigerated prior to metal analysis using ICP-OES.

The percent metal removal and concentration of metals adsorbed on the zeolites were calculated using the following equations:

$$\%\,\text{metal removal} = [(C_o - C_e)/C_e] \times 100 \tag{1}$$

where C_o and C_e are initial and final metal concentrations (mg/l), respectively, and

$$q_e = (C_o - C_e/V/m) \tag{2}$$

where q_e is the final concentration of metals adsorbed by the zeolites (mg/g), m is the mass of zeolite (g) and V is the volume of solution (l).

2.5. Simultaneous disinfection and metal adsorption experiments

To investigate the performance of the zeolites in mixed solutions (*E. coli*-Pb; *E. coli*-Cd; *E. coli*-Zn) 0.2 g of zeolite was added to 50 ml of solution containing *E. coli* (106–108 CFU/ml) and metals (0.5 mg/l) (to represent extreme contamination levels) at pH 5 to prevent metal precipitation. The solutions including controls with no zeolites were shaken for 0–60 min at room temperature and enumeration of viable bacteria was done using the membrane filtration method, while the remaining volume of samples were filtered, acidified to pH < 2 and refrigerated prior to ICP-OES analysis. All experiments were done in duplicates and mean values were used for calculations.

3. Results and discussion

3.1. Characterization of zeolites

The chemical composition of the zeolites before and after silver modification obtained from EDX analysis (Table 1) showed that the zeolite is a potassium-clinoptilolite and confirmed the presence of silver ions in the AgZ framework. The SEM images in Fig. 1 show the typical highly porous structure and platy crystals [17] of the zeolites with no changes observed in the surface properties after silver modification.

3.2. Disinfection performance

The disinfection efficiency of the AgZ against *E. coli* was investigated as a function of contact time. As shown in Fig. 2, there was a rapid reduction in the number of viable cells in the AgZ treated samples as the contact time increased with 100% disinfection achieved after 30 min of contact. The disinfection process may be described as follows:

$$\begin{aligned} & n\text{Ag-Z}_{(\text{zeolite})} + (\text{H}_2\text{O} + E.\ coli)_{(\text{solution})} \\ & \quad \longrightarrow (n - x)\text{Ag-Z}_{(\text{zeolite})} + (\text{Ag}^+ + E.\ coli)_{(\text{solution})} \end{aligned} \tag{3}$$

(where n = silver ions in the zeolite; x = silver ions released in the solution).

The amount of silver released into solution from the AgZ during the disinfection process was also determined. It can be seen, that as the concentration of silver released into the solution increased, there was a reduction in the bacterial count. The amount of silver released into the solution after 30 min of contact when no viable colonies were detected was 1.10 mg/l. There was continued release of silver after all the bacterial cells were eliminated possibly due to the exchange of Ag^+ ions for H^+ ions present in the solution. The final concentration of silver in the solution after 60 min of contact was 2.64 mg/l. No antibacterial action was observed in the NZ treated solutions [11–13,15]. In the control solutions, a slight reduction in the bacterial population was observed after 60 min of contact due to the absence of nutrients necessary for bacterial growth.

Table 1
Chemical composition of AgZ and NZ

Oxide (%)	NZ	AgZ
SiO_2	74.08	70.47
Al_2O_3	11.43	10.24
Fe_2O_3	2.43	1.76
Na_2O	1.71	0.35
CaO	1.41	0.64
MgO	0.85	0.55
K_2O	3.63	3.16
Ag_2O	–	9.44

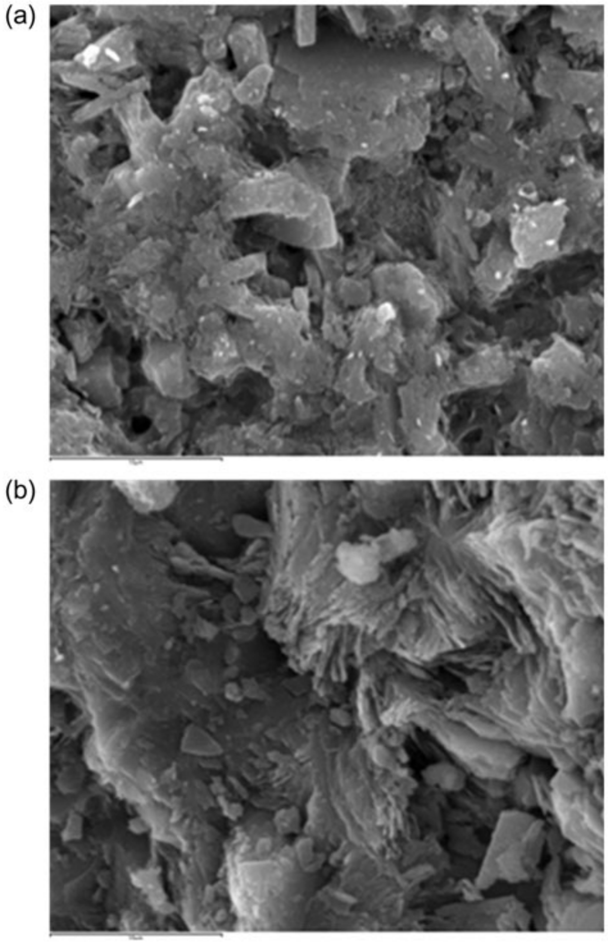

Fig. 1. SEM images of (a) AgZ and (b) NZ.

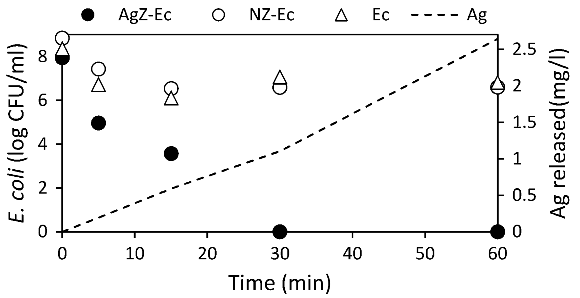

Fig. 2. Disinfection of *E. coli* and amount of silver released from AgZ (mg/l).

These results suggest that the death of *E. coli* is a function of the amount of silver ions released into the solution from AgZ during treatment as reported in other studies [10–13, 15]. The antibacterial action of silver (as illustrated in Fig. 3) occurs when bacterial cells come in contact with silver zeolites suspended in aqueous solutions. The silver ions released from the zeolite framework penetrate the cell wall resulting in detachment of the cytoplasmic membrane and the deposition of dense granules of silver ions in the cell, DNA molecules and around the cell wall. The silver ions bind to and damage the cell DNA resulting in inactivation of proteins and inhibition of essential enzymes. These cause irreversible damage resulting in impaired ability to replicate and cell death [14,15,18,19].

3.3. *Metal removal efficiency*

The metals removal performance of the zeolites was investigated and their corresponding removal efficiencies are shown in Fig. 4. The removal efficiencies for Pb, Cd and Zn were 91, 92 and 85% for AgZ and 82, 68 and 57%, respectively, for NZ. Table 2 shows the equilibrium metal adsorption capacities under the experimental conditions in this study. From the NZ removal results, the order of selectivity towards metal ions was Pb > Cd > Zn as observed in other studies [4,20–22]. However, silver modification of NZ resulted in enhanced ion exchange properties due to the replacement of some less exchangeable potassium ions present in the zeolite as-received by more exchangeable silver ions. Potassium ions are the least exchangeable ions due to their coordination and location (strong bonding sites) within the zeolite framework [23]. The mechanisms involved in metal uptake were ion exchange and adsorption. The agitation of zeolites in metal solutions resulted in rapid mass transfer of metal ions from the bulk liquid to the external zeolite surfaces and intraparticle diffusion within the pores and internal framework. Metal removal occurred due to ion exchange between the metal ions and the exchangeable cations (including the silver ions) present within the zeolite framework and adsorption on the outer and internal mineral surfaces [1,2,24].

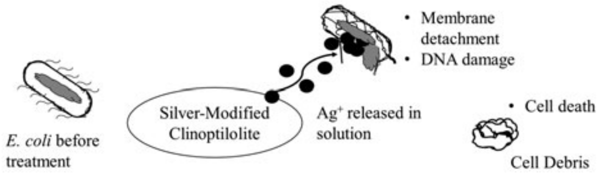

Fig. 3. Antibacterial activity of silver-modified clinoptilolite.

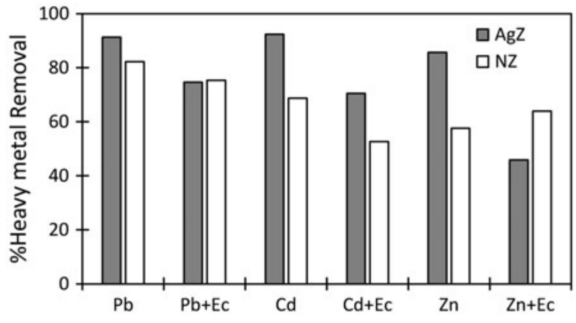

Fig. 4. Percent heavy metal removal from single metal and *E. coli*-metal solutions by AgZ and NZ.

Table 2
Maximum adsorption capacities of AgZ and NZ (initial metal concentration = 0.5 mg/l)

Metals	Solutions	NZ (mg/g)	AgZ (mg/g)
Lead	Pb	0.10	0.11
	Pb (*E. coli*-Pb)	0.10	0.10
Cadmium	Cd	0.09	0.12
	Cd (*E. coli*-Cd)	0.08	0.09
Zinc	Zn	0.07	0.11
	Zn (*E. coli*-Zn)	0.07	0.08

3.4. *Simultaneous disinfection and metal removal efficiency*

The performance of the zeolites in mixed solutions (*E. coli*-Pb, *E. coli*-Cd and *E. coli*-Zn) was investigated. Fig. 5 shows the removal of *E. coli* from mixed solutions as a function of time. As observed in the *E. coli* removal results (Fig. 2), no viable cells were detected in the AgZ treated solutions after 30 min of treatment suggesting a total disinfection. In the NZ treated solutions, there was a reduction in bacterial cells possibly due to the toxic effects of the metal ions in solutions [25–27]. In the control solutions, the observed decrease in bacterial cells can be attributed to the combined effects of starvation and metal toxicity as shown in Fig. 5. The presence of bacteria in the solutions affected the metal removal performance of the zeolites with reduced metal uptake in the mixed solutions (Fig. 4). The removal efficiencies for Pb, Cd and Zn were 75, 71 and 45%, respectively, for AgZ and 75, 52 and 63%, respectively, for NZ. Hence, a proportion of metal ions were not adsorbed by the zeolites but taken up by bacterial cells resulting in cell death and less silver being released into solutions (1.61, 2.11 and

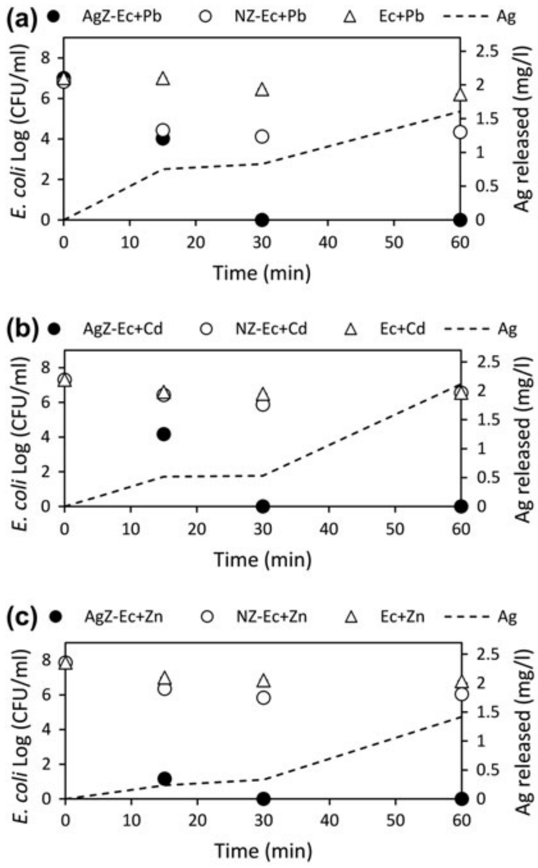

Fig. 5. Disinfection of *E. coli* in (a) *E. coli*-Pb, (b) *E. coli*-Cd and (c) *E. coli*-Zn solutions and amount of silver released from AgZ (mg/l).

1.42 mg/l for *E. coli*-Pb, *E. coli*-Cd and *E. coli*-Zn solutions, respectively). However as expected, the removal efficiency was higher for the *E. coli*-Pb due to the zeolite's higher selectivity towards lead ions. The simultaneous removal processes may be described as follows:

$$n\text{Ag-Z}_{(\text{zeolite})} + (\text{H}_2\text{O} + E.\ coli + \text{Me}^{2+})_{(\text{solution})} \longrightarrow (n - x)\text{Ag} + 2\text{Me-Z}_{(\text{zeolite})} + (\text{Ag}^{+} + E.\ coli)_{(\text{solution})} \tag{4}$$

(where Me = Metal ion (Pb^{2+}, Cd^{2+}, Zn^{2+})).

These results indicate that the antibacterial characteristics observed in the AgZ treated mixed solutions, although mainly due to the antibacterial action of the silver ions released into the solutions, was enhanced by the toxic metal component of the solutions. It was observed that the pH of the treated solutions increased from 5 to 5.8–6.6 after treatment due mainly to the uptake of H^+ ions from the solutions and zeolite hydrolysis [3].

The potential recovery of the silver ions is currently being investigated using multistage column experiments with the view to being used for subsequent enhancement of the as-received zeolites. This is being achieved by adjusting the pH of the AgZ treated effluent to the optimum pH for silver adsorption and passing the solution through an appropriately sized bed of non-modified natural zeolites (NZ) to achieve maximum uptake of silver ions by adsorption/ion exchange. In the process, the NZ column is being modified for future use as a new AgZ column.

4. Conclusions

The use of AgZ was investigated for its combined metal removal and antibacterial characteristics. AgZ exhibited excellent antibacterial properties with complete elimination of bacteria within 30 min of contact time and high metal removal efficiencies (up to 92%) for metals present in solutions. In the mixed solutions (metals + *E. coli*), the interaction between the metal ions and bacterial cells resulted in a slight decrease in metal removal efficiencies due to the retention of metal ions by the cell structure. The concentration of silver released into the solutions was also monitored and in excess of tolerable levels highlighting the need for an efficient method of silver recovery and reuse currently being investigated. The NZ did not exert any antibacterial activity. These findings indicate that silver-modified clinoptilolite has potential for the combined removal of *E. coli* and heavy metals from contaminated aqueous streams.

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