Article

Removal Performance of Faecal Indicators by Natural and Silver-Modified Zeolites of Various Particle Sizes under Dynamic Batch Experiments: Preliminary Results

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Abstract: One of the oldest and most promising applications of natural zeolites (NZs) is in water and wastewater treatment processes. Modified zeolites (MZs), with improved ion exchange and adsorption capacities, have been extensively applied to the removal of pollutants from aqueous solutions. However, the application of MZs in pathogens or indicator organisms has not been extensively explored. This study examines the effect of both natural Greek zeolite (NZ), with a clinoptilolite content of up to 85% (OLYMPUS SA-INDUSTRIAL MINERALS), and modified Greek zeolite through incorporation with silver ions (Ag-MNZ), on the survival of two selected faecal indicator bacteria (Escherichia coli and Enterococcus faecalis). A series of dynamic batch experiments with a slow agitation of 12 rpm were conducted at a constant ambient temperature (22°C) in order to examine the inactivation of the above bacteria by NZ and Ag-MNZ. It was found that the Ag-MNZ resulted in a much higher reduction in the bacterial numbers when compared to the NZ and the control (absence of zeolites). Moreover, the reduction in bacterial numbers was affected by NZ particle size, with higher removal rates observed for coarse (1–3 mm) than for fine (0–1 mm) NZ. Finally, the E. faecalis was found to be more resistant than E. coli to Ag-MNZ.

Keywords: Escherichia coli; Enterococcus faecalis; natural zeolites; modified zeolites; silver ions; inactivation

1. Introduction

Water bodies, both surface and groundwater, may be contaminated by waterborne pathogens that pose significant health risks to humans and cause millions of deaths per year [1–4]. Therefore, the efficient removal of pathogens is urgently needed to guarantee the safety of water. Chlorination, the most used conventional water disinfection method in most countries, is ineffective against highly resistant bacteria and also forms carcinogenic disinfection by-products (DBPs). Thus, there is a necessity to develop a cost-effective, environmentally neutral disinfection solution with minimized chemical and energy footprint for pathogen removal.

Natural zeolites (NZs) are crystalline, hydrated alumina silicates of alkali and alkaline earth cations, with large deposits in many parts of the world. Their large reserves, the high ion exchange capacity and low market price make zeolites good potential materials for water and wastewater treatment and soil remediation [5–8]. Loosely bound cations (Na\(^+\), K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\)) in zeolites can be easily exchanged with other cations such as silver ions.

Silver in the ionic form of Ag\(^+\) (soluble and ion doped or exchanged into organic and inorganic materials) or Ag\(^0\) in cluster formations (nanoparticles) exhibit good antibacterial properties [9–15]. Silver incorporated into other materials such as synthetic zeolites [16–27] and natural zeolites [27–33] seems to be the most common and low-cost way due to their...
easily handling, non-toxicity, and their ability to control the long-term release rate in bioactive applications [34].

The antibacterial behavior of Ag-zeolites on different microorganisms has been studied mainly for health and food field applications [35–37]. Despite previous research on the antibacterial effects of Ag-modified natural zeolites (Ag-MNZs), how bacterial inactivation in aqueous media occurs by Ag-MNZs of various particle sizes has not been explored.

The primary scope of this study is to compare the removal efficiency of NZs and Ag-MNZs against two water quality and wastewater process indicator bacteria. The most commonly tested indicators are total coliforms, faecal coliforms, and faecal indicator bacteria (FIB) such as Escherichia coli (E. coli), and enterococci (e.g., E. faecalis). The selected Gram-negative E. coli and Gram-positive E. faecalis are abundant in human and animal faeces and are also found in sewage, treated effluent, and all natural waters. Hence, a comprehensive understanding of the key processes of their removal is essential for public health protection through the development of more effective water purification and disinfection strategies.

2. Materials and Methods

2.1. Natural Zeolite

Natural zeolite is a low-cost mineral found worldwide in large amounts. The natural Greek zeolite (Olympus Industrial Minerals S.A.) is collected from a deposit in the northern region of Greece. The major component (>85%) is clinoptilolite in multicationic form [(Na,K,Ca)6(Si,Al)36O72·20H2O] associated with impurities such as mica/illite, plagioclase feldspar, and quartz. Note that the Na⁺ cation in the structure of natural clinoptilolite, due to the similar hydrated ionic radii, is preferable in an exchange with the hydrated Ag⁺ ion to Ca²⁺, K⁺, and Mg²⁺ cations [38,39]. Moreover, the selectivity for the clinoptilolite was found to follow the sequence K⁺ > NH₄⁺ > Ag⁺ > Pb²⁺ > Na⁺ > Ca²⁺ > Li⁺ [40]. Natural Greek zeolite (NZ) was obtained in ground form and was then sieved to different fractions, of sizes 0–1 (fine) and 1–3 mm (coarse). Afterwards, the zeolite was thoroughly cleaned with deionized water (DIW) in order to eliminate any remaining dust and dried at 60 °C for desiccator storing.

2.2. Modification of Natural Zeolite with Silver

The Ag⁺ modification of natural zeolite was performed using silver nitrate (AgNO₃) and the ion exchange method outlined by Boschetto et al. [41]. Briefly, 3 g of natural zeolite was added to 50 mL of AgNO₃ aqueous solution (0.25% w/v) at pH 5 ± 0.2 (to prevent metal precipitation) and shaken at 300 rpm for 24 h in tubes wrapped with aluminium foil so that the formation of black silver oxide was avoided while achieving the maximum exchange of silver ions into the zeolites. Note that a pH value greater than 7.5–8 results in dark samples because Ag⁺ in the zeolite turns into Ag⁰ [42]. Then, the Ag-exchanged zeolite solution was centrifuged and, after repeated washings with DIW, the zeolite was dried at 60 °C for a duration of 24 h to obtain the silver-modified natural zeolite (0.25%Ag-MNZ). The selectivity of zeolites towards Ag⁺ tends to be higher with an increased Si/Al ratio [43].

2.3. Natural and Silver-Modified Zeolite Characterization

The morphologies of selected NZs and Ag-MNZs were examined by a JSM 6300 JEOL Scanning Electron Microscope (SEM) at 20 kV. The energy dispersive X-ray (EDX) analysis of fine (0–1 mm) NZ, coarse (1–3 mm) NZ, fine (0–1 mm) Ag-MNZ, and coarse (1–3 mm) Ag-MNZ was also observed using this instrument. Clearly, the incorporation of Ag⁺ into the structure of Ag-MNZ did not change its morphology and thusly formations of NZ and Ag-MNZ were similar in particle size and appearance (see Figure 1). However, Boschetto et al. (2012) observed crystallinity and specific area loss after Ag incorporation in the structure of the zeolite Y [41]. The results of EDX analyses (data not shown) suggest that the molar ratio Si/Al of the NZ (≈6) does not change notably with Ag exchange. Note
that high concentrations of Ag could cause significant changes in the zeolite structure [41]. SEM images also captured aggregates as finer crystal grains of the clinoptilolite mineral.

![SEM images taken at a magnification of 2500× of (a) 0–1 mm NZ, (b) 0–1 mm Ag-MNZ, (c) 1–3 mm NZ, and (d) 1–3 mm Ag-MNZ.](image)

**Figure 1.** SEM images taken at a magnification of 2500× of (a) 0–1 mm NZ, (b) 0–1 mm Ag-MNZ, (c) 1–3 mm NZ, and (d) 1–3 mm Ag-MNZ.

### 2.4. Bacterial Suspensions Preparation and Enumeration

The two model bacteria used in the batch experiments were the Gram-negative *E. coli* (NCTC 9001) and the Gram-positive *E. faecalis* (NCTC 775). Cultures were stored at −80 °C in growth media enhanced with 80% glycerol. Prior to each batch experiment, the bacteria were cultured on non-selective growth medium (Nutrient Agar) at 37 °C for 48 h. Subsequently, some of the sufficiently formed colonies of the microbial cultures were isolated and transferred to a test tube of sterilized DIW; bacterial concentration in the suspension was calculated based on the 0.5 McFarland turbidity scale, according to which, 0.1 optical absorbance at 600 nm equals to a concentration of 10^8 cfu/mL. Optical density measurements were conducted using a UV-visible spectrophotometer (U-2001, Hitachi, Tokyo, Japan). The dense bacterial suspensions were diluted to obtain the initial bacterial concentrations for the batch experiments. For the calculation of the bacteria concentrations, serial dilution of the samples was performed and aliquots of 100 µL were plated (in duplicates) on the surface of selective growth media: Harlequin Chromogenic Coliform Agar (NEOGEN NCM 1005A) and Slanetz and Bartley Agar (LAB166) for the growth of *E. coli* and *E. faecalis*, respectively. The plates were incubated at 37 °C for 48 h and the colonies were counted. Reliable dilutions for quantification were considered those that resulted in a reasonable number (30–300) of distinct colonies to count. Then, the concentration of bacteria in the sample was measured by taking into account the sample dilution and the volume plated out on the growth media, and given in colony-forming units per milliliter (cfu/mL) [44].

### 2.5. Batch Inactivation Experiments

For bacteria inactivation experiments with the NZ and the Ag-MNZ, a standard procedure in the batch process was followed. In the batch process a solution of synthetic water inoculated with bacteria (*E. coli, E. faecalis*) containing a certain amount of NZ or Ag-MNZ (5 g/L) was used. The procedure was implemented under a constant ambient temperature and hydrodynamic conditions, while samples were obtained at specified intervals. Tubes with a volume of 50 mL were completely filled in a gentle manner with the above synthetic water inoculated with bacteria of two tested initial concentrations, 10^6 and
10^5 cfu/mL, so that no air remained upon their closure with caps. The tubes were attached onto a rotator. Then, a slow agitation of 12 rpm, associated with the dynamic conditions, was chosen in order to maximize the exposure of bacteria to NZ or Ag-MNZ.

For each experiment, 20 tubes divided into two sets were used. The first set of 10 tubes (controls) contained a 50 mL suspension of bacteria without NZ or Ag-MNZ in order to observe the time-dependent bacterium inactivation. The second set of 10 tubes (reactors) contained a 50 mL mixed suspension of bacteria with NZ or Ag-MNZ in order to observe any changes in bacterium inactivation provoked by the presence of zeolites. At various time intervals over a 1 h time period, a tube from each set of tubes was selected for sampling. The supernatant was sampled (a 2 mL collected sample) to determine the remaining bacteria concentration. Figure 2 illustrates the batch experimental procedure.

**Figure 2.** Graphical representation of the dynamic batch experimental procedure. The control tubes were filled with bacteria suspension and the reactor tubes were filled with bacteria suspension as well as natural zeolite or Ag-modified natural zeolite. All tubes were attached to a tube rotator at 12 rpm and 22 °C.

### 2.6. Theoretical Considerations

The log reduction in bacterial concentrations after the experimental time period was obtained by applying the following expression [45]:

$$\log(\text{Reduction}) = \log_{10}\left(\frac{C_{\text{total,0}}}{C_{\text{total}(t)}}\right) = \log_{10}C_{\text{total,0}} - \log_{10}C_{\text{total}(t)} \quad (1)$$

where $C_{\text{total}}$ [cfu/mL] is the total bacterial concentration in the suspension at time $t$ and $C_{\text{total,0}} = C_{\text{total}(t=0)}$ is the initial total concentration of bacteria.

Additionally, the log reduction was converted to percent reduction ($P$) as follows:

$$P(\%) = (1 - 10^{-\log(\text{Reduction})}) \times 100 \quad (2)$$

Moreover, the below pseudo-first-order mathematical expression, accounting for time-dependent inactivation, was used to describe the experimental bacterial inactivation data [46–48]:

$$\frac{dC_{\text{total}}(t)}{dt} = -\lambda(t)C_{\text{total}}(t) \quad (3)$$
where \( \lambda \) [1/d] is the inactivation rate coefficient of bacteria which can be written as a function of time:

\[
\lambda(t) = -\lambda_0 e^{-\alpha t}
\]  

(4)

where \( \lambda_0 \) [1/d] is the initial inactivation rate coefficient, and \( \alpha \) [1/d] is the resistivity coefficient.

The solution to Equation (3) is given as:

\[
\ln \left( \frac{C_{\text{total}}(t)}{C_{\text{total},0}} \right) = -\frac{\lambda_0}{\alpha} \left( e^{-\alpha t} - 1 \right)
\]

(5)

If \( \lambda \) is time independent \( \lambda(t) = \lambda \), then the solution to Equation (3) is:

\[
\ln \left( \frac{C_{\text{total}}(t)}{C_{\text{total},0}} \right) = -\lambda t
\]

(6)

The unknown inactivation parameters \( \lambda \), \( \lambda_0 \) and \( \alpha \) were estimated using the autonomous multipurpose fitting software: ColloidFit [49].

3. Results and Discussion

3.1. Bacterial Inactivation in the Presence of NZs

Figure 3 presents the inactivation results of both bacteria (E. coli, E. faecalis) in the absence (controls) and presence (reactors) of either fine (0–1 mm) or coarse (1–3 mm) NZs under dynamic conditions at 22 °C for two different initial bacteria concentrations (10^5 and 10^6 cfu/mL). Note that slight variations in the initial concentration of the bacteria suspensions present in each tube may result in normalized bacterial concentrations greater than unity. The experimental results show that in the absence of NZs, in most cases examined, the inactivation rates for both bacteria were higher for the higher initial bacterial concentration compared to the inactivation rates for bacteria with the lower initial concentration (Table 1, Figure 4). Although previous studies showed that bacteria inactivation rates decrease with an increase in initial bacteria concentration [50], in this study, such a clear trend was not observed.

Moreover, similar inactivation rates were observed in reactor and control tubes (absence of NZs) for both bacteria (E. coli, E. faecalis), suggesting that low concentration (5 g/L) of both fine (0–1 mm) and coarse (1–3 mm) NZs only slightly affects bacteria inactivation (no antibacterial effect) for both initial bacteria concentrations (10^5 and 10^6 cfu/mL). However, in most cases examined, higher inactivation rates were observed in the case of coarse rather than fine NZs.

Table 1. Fitted inactivation parameter values (\( \lambda \), \( \lambda_0 \), and \( \alpha \)) of E. coli and E. faecalis for the two initial concentrations (10^6 and 10^5 cfu/mL) under experimental conditions.
| Bacteria | Experimental Case | Absence/Presence of NZ or Ag-MNZ | Bacteria Initial Concentration | $\lambda$ (min$^{-1}$) | $\lambda_0$ (min$^{-1}$) | $\alpha$ (min$^{-1}$) |
|----------|------------------|----------------------------------|-----------------------------|----------------|----------------|----------------|
| *E. faecalis* | | | | | | |
| Controls | 10$^6$ cfu/mL | 3.07 $\times$ 10$^{-2}$ $\pm$ 0.017 | 18.3 $\times$ 10$^{-2}$ $\pm$ 0.194 | 0.015 $\pm$ 0.171 |
| Reactors | 10$^5$ cfu/mL | 1.8 $\times$ 10$^{-2}$ $\pm$ 0.007 | 4.18 $\times$ 10$^{-2}$ $\pm$ 0.007 | 0.518 $\pm$ 0.153 |
| *E. coli* | | | | | | |
| Controls | 10$^6$ cfu/mL | 4.57 $\times$ 10$^{-2}$ $\pm$ 0.022 | 30.9 $\times$ 10$^{-2}$ $\pm$ 0.049 | 0.916 $\pm$ 0.311 |
| Reactors | 10$^5$ cfu/mL | 1.70 $\times$ 10$^{-2}$ $\pm$ 0.009 | 20.56 $\times$ 10$^{-2}$ $\pm$ 0.102 | 0.078 $\pm$ 0.117 |
| *E. faecalis* | | | | | | |
| Controls | 10$^6$ cfu/mL | 2.28 $\times$ 10$^{-2}$ $\pm$ 0.016 | 35.59 $\times$ 10$^{-2}$ $\pm$ 0.011 | 0.988 $\pm$ 0.066 |
| Reactors | 10$^5$ cfu/mL | 1.81 $\times$ 10$^{-2}$ $\pm$ 0.009 | 10.91 $\times$ 10$^{-2}$ $\pm$ 0.021 | 1.142 $\pm$ 0.511 |
| *E. coli* | | | | | | |
| Controls | 10$^6$ cfu/mL | 4.37 $\times$ 10$^{-2}$ $\pm$ 0.076 | 70.43 $\times$ 10$^{-2}$ $\pm$ 0.090 | 0.191 $\pm$ 0.045 |
| Reactors | 10$^5$ cfu/mL | 3.62 $\times$ 10$^{-2}$ $\pm$ 0.008 | 13.84 $\times$ 10$^{-2}$ $\pm$ 0.288 | 1.475 $\pm$ 8.4 |
| *E. faecalis* | | | | | | |
| Controls | 10$^6$ cfu/mL | 7.91 $\times$ 10$^{-2}$ $\pm$ 0.009 | 11.16 $\times$ 10$^{-2}$ $\pm$ 0.008 | 0.292 $\pm$ 0.038 |
| Reactors | 10$^5$ cfu/mL | 4.09 $\times$ 10$^{-2}$ $\pm$ 0.013 | 16.07 $\times$ 10$^{-2}$ $\pm$ 0.012 | 0.471 $\pm$ 0.059 |
| *E. coli* | | | | | | |
| Controls | 10$^6$ cfu/mL | 5.39 $\times$ 10$^{-2}$ $\pm$ 0.091 | 49.25 $\times$ 10$^{-2}$ $\pm$ 0.068 | 0.344 $\pm$ 0.081 |
| Reactors | 10$^5$ cfu/mL | 16.34 $\times$ 10$^{-2}$ $\pm$ 0.013 | 43.25 $\times$ 10$^{-2}$ $\pm$ 0.023 | 0.142 $\pm$ 0.016 |
| *E. faecalis* | | | | | | |
| Controls | 10$^6$ cfu/mL | 1.42 $\times$ 10$^{-2}$ $\pm$ 0.008 | 12.91 $\times$ 10$^{-2}$ $\pm$ 0.014 | 1.326 $\pm$ 0.357 |
| Reactors | 10$^5$ cfu/mL | 1.95 $\times$ 10$^{-2}$ $\pm$ 0.004 | 4.70 $\times$ 10$^{-2}$ $\pm$ 0.009 | 0.629 $\pm$ 0.219 |
| *E. coli* | | | | | | |
| Controls | 10$^6$ cfu/mL | 15.82 $\times$ 10$^{-2}$ $\pm$ 0.071 | 75.14 $\times$ 10$^{-2}$ $\pm$ 0.276 | 0.035 $\pm$ 0.076 |
| Reactors | 10$^5$ cfu/mL | 100.92 $\times$ 10$^{-2}$ $\pm$ 0.142 | 327.82 $\times$ 10$^{-2}$ $\pm$ 0.256 | 0.527 $\pm$ 0.074 |
| *E. faecalis* | | | | | | |
| Controls | 10$^6$ cfu/mL | 6.45 $\times$ 10$^{-2}$ $\pm$ 0.017 | 25.8 $\times$ 10$^{-2}$ $\pm$ 0.040 | 15.269 $\pm$ 6.665 |
| Reactors | 10$^5$ cfu/mL | 6.40 $\times$ 10$^{-2}$ $\pm$ 0.014 | 8.01 $\times$ 10$^{-2}$ $\pm$ 0.029 | 0.136 $\pm$ 0.106 |
| *E. coli* | | | | | | |
| Controls | 10$^6$ cfu/mL | 26.03 $\times$ 10$^{-2}$ $\pm$ 0.036 | 36.0 $\times$ 10$^{-2}$ $\pm$ 0.040 | 0.447 $\pm$ 0.087 |
| Reactors | 10$^5$ cfu/mL | 28.35 $\times$ 10$^{-2}$ $\pm$ 0.026 | 16.44 $\times$ 10$^{-2}$ $\pm$ 0.036 | 0.083 $\pm$ 0.053 |
Figure 3. Experimental inactivation data of *E. faecalis* (a–d) and *E. coli* (e–h) removal by fine (0–1 mm) NZ (a–g) and coarse (1–3 mm) NZ (b–h) in the absence (empty symbols) and the presence (filled symbols) of natural zeolites. Bacterial initial concentrations: $10^6$ and $10^5$ cfu/mL.
Table 1. Fitted inactivation parameter values (controls) and presence (reactors) of either fine (0–1 mm) or coarse (1–3 mm) 0.25% Ag-MNZs, low compared to high initial bacteria concentrations (10^5 and 10^6 cfu/mL) under experimental conditions.

| C_0 (cfu/mL) | E. coli (a,b) | E. faecalis (c,d) |
|--------------|---------------|-------------------|
| 10^5         |               |                   |
| 10^6         |               |                   |

Note: The table data is not fully visible in the image.

3.2. Bacterial Inactivation in the Presence of Ag-MNZs

Figure 4 shows the inactivation results of both bacteria (E. coli, E. faecalis) in the absence (controls) and presence (reactors) of either fine (0–1 mm) or coarse (1–3 mm) 0.25% Ag-MNZs under dynamic conditions at 22 °C for two different initial bacteria concentrations (10^5 and 10^6 cfu/mL). Clearly, higher inactivation rates are observed in the presence of 0.25% Ag-MNZs than NZs for both bacteria (E. coli, E. faecalis) and the two initial bacteria concentrations (10^5 and 10^6 cfu/mL) (Table 1, Figure 4). Similar inactivation rates were observed for the two initial concentrations under coarse (1–3 mm) 0.25% Ag-MNZs. In the presence of fine (0–1 mm) 0.25% Ag-MNZs, low compared to high initial bacteria concentrations yielded higher inactivation rates. Similar results for E. coli removal by natural zeolites and Mg^{2+}-modified zeolites (MMZs) were found by Sang-Woo et al. (2016) [51]. The authors reported that increased initial E. coli concentration causes the zeolite adsorption capacity to decrease. An enormous E. coli adsorption capacity of MMZs compared to that of natural zeolite for different initial E. coli concentration was also observed. Note that zeolite morphology and particle size may alter bacteria-zeolite interactions and consequently the extent of bacterial inactivation. Zeolites with a smaller particle size are expected to result in faster bacterial inactivation due to faster ion exchange and release (shorter diffusion length) [27]. However, in this study no clear trend was observed. Note that significant variations in bacteria inactivation results among the replicates suggest the creation of bacterial aggregates. However, the bacterial inactivation data collected in this study cannot be compared directly with the results from previous studies, because of the differences in experimental conditions (e.g., silver modification of zeolites, zeolite and bacteria concentrations, reactor geometries, aqueous solutions, cultivation conditions etc.). Moreover, slight changes in water chemistry may result in different agglomeration and sedimentation behaviour, as well as in the toxic activity of Ag-MNZs.
Figure 5. Experimental inactivation data of *E. faecalis* (a–d) and *E. coli* (e–h) removal by fine (0–1 mm) Ag-MNZ (a–g) and coarse (1–3 mm) Ag-MNZ (b–h) in the absence (empty symbols) and the presence (filled symbols) of zeolites. Bacterial initial concentrations: $10^6$ and $10^5$ cfu/mL.

3.3. Bacteria Log Reduction

Figure 6 presents a graphical representation of the bacteria log reduction within a 1-h time period for all cases considered in this study. The calculated log and percent reduction values are presented in Table 2. In most cases examined under NZs, higher log reduction values were observed in the case of a higher initial concentration for both bacteria (*E. coli*, *E. faecalis*). However, in both control and reactor tubes within 1 h of the experiment, no substantial reduction in the bacteria’s initial concentrations were observed. In the presence of fine (0–1 mm) NZ, the reduction in *E. faecalis* by $0.12 \pm 0.08 \log \text{ cfu/mL}$ (≈23.58%) and $0.09 \pm 0.02 \log \text{ cfu/mL}$ (≈18.72%) was similar to that in *E. coli* ($0.15 \pm 0.08 \log \text{ cfu/mL}$, ...
~29.21% and 0.11 ± 0.14 log cfu/mL, ~22.52%) for 10^6 and 10^5 cfu/mL initial concentrations, respectively. In the presence of coarse (1–3 mm) NZ, the E. coli population decreased by 0.26 ± 0.05 log cfu/mL (~44.87%) and 0.15 ± 0.11 log cfu/mL (~28.98%) within a 1 h time period for the 10^6 and 10^5 cfu/mL initial concentrations. E. faecalis was less sensitive to the presence of coarse (1–3 mm) NZ with a reduction of 0.10 ± 0.05 log cfu/mL (~21.04%) and 0.09 ± 0.07 log cfu/mL (~19.49%) for 10^6 and 10^5 cfu/mL initial concentrations, respectively.

The silver ions incorporated into NZs enhanced the bacterial population reduction in the presence of both fine (0–1 mm) and coarse (1–3 mm) 0.25% Ag-MNZs for both initial bacterial concentrations (10^6 and 10^5 cfu/mL) and both bacteria (Figure 6c,d,g,h). Clearly, the presence of fine (0–1 mm) 0.25% Ag-MNZs resulted in a greater population reduction for E. coli (1.53 ± 0.07, ~97.07% and 1.97 ± 0.63 log cfu/mL, ~98.93%) and for E. faecalis (~1.50 ± 0.56 and 2.96 ± 0.24 log cfu/mL) for 10^6 and 10^5 cfu/mL initial concentrations, respectively. Coarse (1–3 mm) Ag-MNZs enhanced both E. coli (~1.97 ± 1.01 and 2.35 ± 0.84 log cfu/mL) and E. faecalis population reduction (~1.56 ± 0.11 and 1.79 ± 0.14 log cfu/mL) for the two above initial concentrations. Note that higher log reduction for both bacteria populations was observed in the case of lower initial bacterial concentration.

Top and Ülkü (2004) showed that the antibacterial activity of Ag⁺-clinoptilolite did not increase with increasing amounts of Ag⁺ in the zeolite, probably due to the formation of metallic Ag at high Ag⁺ concentrations [29]. Akhigbe et al. (2014) reported a significant reduction in E. coli growth by Ag⁺-modified clinoptilolite (4.34 wt%) with a 10 log_{10} reduction in 30 min [28]. The bactericidal action of silver-modified zeolite in the literature has been attributed to two mechanisms. The first one is based on the silver ion itself being released from the zeolite [52] and the second one on reactive oxygen species (ROS) generated from silver in the solid matrix [27]. Milenkovic et al. (2017) found that the bactericidal effect of Ag-NZs toward E. coli could be attributed not only to released Ag⁺ ions but also to Ag-Z itself [32]. Moreover, in most cases examined in this study, greater population reduction was observed for E. coli than E. faecalis, probably due to the higher resistance of Gram-positive bacteria (E. faecalis) than Gram-negative bacteria (E. coli) caused by the thicker peptidoglycan layer.
Table 2. Calculated log and percent reduction values of *E. coli* and *E. faecalis* in the presence of NZ and 0.25% Ag-MNZ within the period of 1 h under the experimental conditions.

| Experimental Conditions | Duration: 1 h | Controls | Reactors | Controls | Reactors |
|-------------------------|--------------|----------|----------|----------|----------|
| Initial Bacterial Concentrations: | | | | | |
| | E. coli | | | E. faecalis | | |
| | log(Reduction) | P (%) | log(Reduction) | P (%) | log(Reduction) | P (%) |
| Coarse (1–3 mm) NZ | | | | | |
| 10⁶ cfu/mL | 0.12 ± 0.04 | 24.14 ± 10.49 | 0.15 ± 0.08 | 29.21 ± 6.86 | 0.09 ± 0.07 | 19.24 ± 8.40 | 0.12 ± 0.08 | 23.58 ± 17.46 |
| 10⁵ cfu/mL | 0.17 ± 0.04 | 32.39 ± 11.73 | 0.11 ± 0.14 | 22.52 ± 13.38 | 0.07 ± 0.07 | 14.89 ± 7.46 | 0.09 ± 0.02 | 18.72 ± 3.42 |
| Fine (0–1 mm) 0.25% Ag-MNZ | | | | | |
| 10⁶ cfu/mL | 0.22 ± 0.12 | 39.74 ± 15.01 | 1.53 ± 0.07 | 97.07 ± 0.49 | 0.06 ± 0.04 | 12.37 ± 8.80 | 1.50 ± 0.56 | 96.81 ± 1.55 |
| 10⁵ cfu/mL | 0.16 ± 0.04 | 30.82 ± 6.23 | 1.97 ± 0.63 | 98.93 ± 2.35 | 0.03 ± 0.02 | 6.67 ± 6.86 | 2.96 ± 0.24 | 99.89 ± 0.09 |
| Coarse (1–3 mm) 0.25% Ag-MNZ | | | | | |
| 10⁶ cfu/mL | 0.3 ± 0.07 | 49.88 ± 17.05 | 1.97 ± 1.01 | 98.93 ± 3.91 | 0.19 ± 0.06 | 35.43 ± 6.03 | 1.56 ± 0.11 | 97.27 ± 0.84 |
| 10⁵ cfu/mL | 0.22 ± 0.05 | 39.74 ± 7.20 | 2.35 ± 0.84 | 99.55 ± 1.17 | 0.16 ± 0.09 | 30.82 ± 11.98 | 1.79 ± 0.14 | 98.28 ± 0.44 |

4. Conclusions

The results of this study show that under dynamic batch conditions, both *E. coli* and *E. faecalis* were removed in greater amounts by Ag-modified NZs than NZs for both particle sizes (0–1 mm and 1–3 mm) employed in this study at 22 °C. Furthermore, the log reduction in both bacteria was found to be dependent on the initial bacteria concentration. Moreover, a higher percent reduction was observed for fine than coarse NZ, while in the case of 0.25% Ag-MNZ, no clear trend was observed. Note that the 0.25% Ag-MNZ reduced both bacteria from 1.5 to 2.96 logs after 1 h. The estimated inactivation rates suggest that bacteria inactivation, in most cases considered in this study, is inversely correlated with zeolite particle size in the presence of 0.25% Ag-MNZs.

The preliminary results obtained in this research stage show the possibility of using NZs or Ag-MNZs of various particle sizes in the removal of pathogens or faecal indicators in water treatment processes. Modified zeolites, with exceptional ion-exchange and sorption properties, have the potential to be effective filtration media. The development of Ag-MNZ-based filters, with enhanced antimicrobial properties, lays the framework for new possibilities in water filtration. However, future work is needed to evaluate the removal of waterborne microbial pathogens by Ag-MNZs in fixed-bed columns under a broad range of conditions including: the Ag-MNZ incorporation method, solution pH, pathogen initial concentration, flow rate, and bed depth. Moreover, the potential of silver recovery for reuse should also be evaluated. Based on the results of further research, it will be possible to economically assess the whole technological process and give a clear opinion on the use of NZ and Ag-MNZ in water treatment processes.

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