



Removal performance of faecal indicators by natural and silver modified zeolites under dynamic batch experiments

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Abstract

The use of natural zeolites (NZs) in waste-water treatment plants is one of the oldest and most promising applications. Modified natural zeolites (MNZs) have shown improved ion exchange and adsorption capacities and have been extensively applied for the removal of pollutants (metal(loid) ions, ammonia etc) from aqueous solutions. However, MNZs application in biological pollutants such as indicator organisms or pathogens has not been extensively explored. This study examines the antimicrobial effect of both natural Greek zeolite (NZ), with clinoptilolite content up to 85% (OLYMPUS SA), and modified Greek zeolite by incorporation with silver ions (Ag-MNZ) on the survival of two selected bacteria. The chosen organisms, *Escherichia coli* and *Enterococcus faecalis*, constitute indicators of fecal contamination in both soils and water. Scanning electron microscopy and energy dispersive X-ray detection (SEM-EDX) were used for the surface morphology and elemental composition of the NZ and Ag-MNZ samples, respectively. A series of dynamic batch experiments were conducted at constant room 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 much higher reduction of the bacterial numbers when compared to the NZ and control (absence of zeolites). Moreover, the reduction in the bacterial numbers was affected by NZ particle size with higher reduction observed for fine (0-1 mm) than coarse (1-3) NZ. Finally, in the most cases examined, *E. faecalis* was found to be more resistant than *E. coli* to Ag-MNZ.

Materials and Methods

Model Bacteria

- Gram (-): *Escherichia (E.) coli* (CRM09001M);
- Gram (+): *Enterococcus (E.) faecalis* (CRM00775M)
- Bacteria initial experimental concentration: 10^3 - 10^6 cfu/mL

Natural Zeolite (NZ) and Ag-Modified Natural Zeolite (Ag-MNZ)

1. Natural zeolites (NZs)

With clinoptilolite content up to 85%

OLYMPUS S.A, Greece



0-1 mm (Fine)



1-3 mm (Coarse)

2. Silver Modified Natural zeolites (Ag-MNZs)

Preparation of Ag-MNZ by ion exchange method

- 3 g of NZ was suspended in 50 mL of AgNO_3 aqueous solution (0.25 w/v) at pH 5 ± 0.2 (to prevent metal precipitation)
- Shaking at 300 rpm for 24 h in a dark room (due to the light sensitivity of silver)
- Centrifugation of Ag-exchanged zeolite solution, washing with deionized water drying overnight at 60 °C, and storage ready for use.

Characterization of NZ and Ag-MNZ

Scanning electron microscopy and energy dispersive X-ray detection (SEM-EDX)

Chemical composition of untreated commercial zeolite:

- NZ 0-1 mm: 69.66% SiO_2 , 10.5% Al_2O_3 , 0.57% CaO , 2.43% K_2O , 1.21% MgO , 0.71% Na_2O , 3.52% Fe_2O_3 , and LOI 11.40%
- NZ 1-3 mm: 66.46% SiO_2 , 10.7% Al_2O_3 , 1.09% CaO , 5.61% K_2O , 1.7% P_2O_5 , 1.56% Na_2O , 1.88% Fe_2O_3 , and LOI 11%

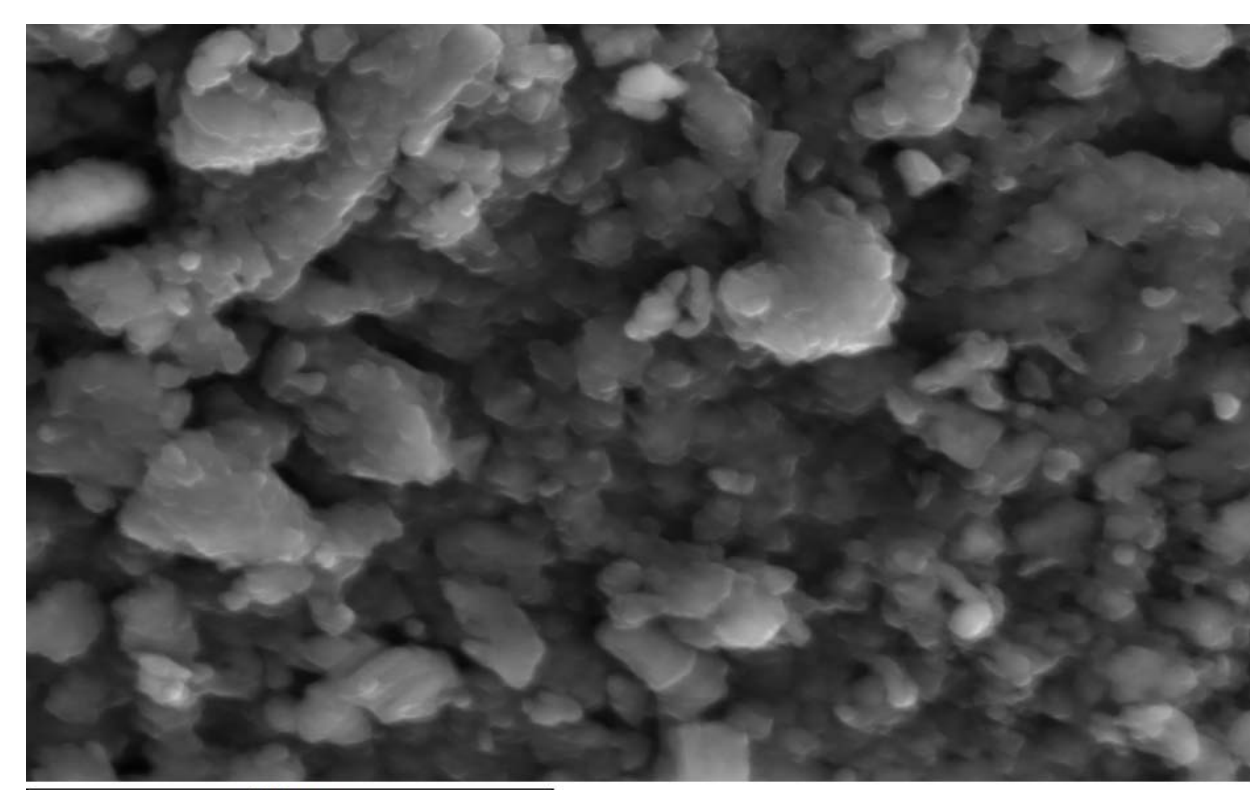
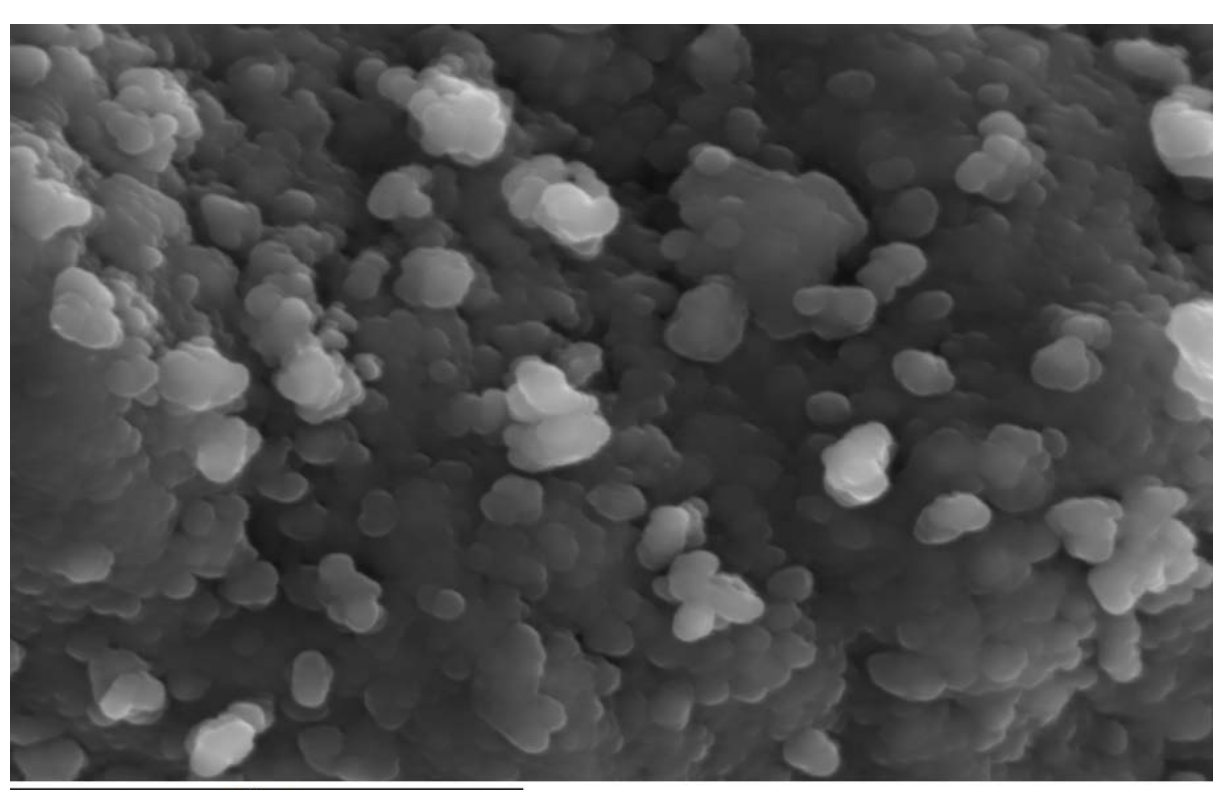
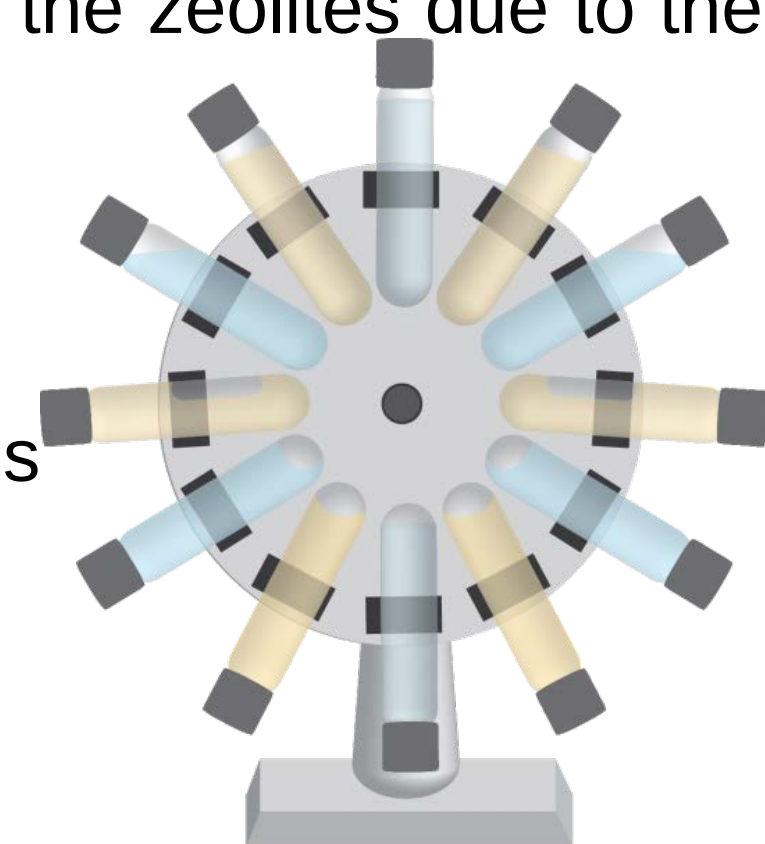


Figure 1: SEM images of (a) untreated commercial 0-1 mm natural zeolite (NZ) and (b) Ag-modified 0-1 mm zeolite (Ag-MNZ)

Experimental Set Up

Batch experiments

- 50-mL tubes
 - 10 tubes (controls) contained 50 mL suspension of bacteria without NZ or Ag-MNZ
 - 10 tubes (reactors) contained 50 mL suspension of bacteria with NZ or Ag-MNZ
- 0.25 g of NZ and Ag-MNZ were suspended in 50-mL solutions of FIB (*E. coli*, *E. faecalis*) in deionized water (10^4 - 10^6 cfu/mL).
- pH 5 ± 0.2 (in order metal precipitation to be avoided)
- Tubes were wrapped with aluminium foil to prevent the formation of black silver oxide on the zeolites due to the light sensitivity of silver ions.
- Dynamic conditions:
 - rotator working at 12 rpm
- Samples:
 - 2 mL at pre-selected time intervals



Results

Bacteria removal

The log reduction of bacterial concentrations within the experimental time period was obtained by applying the following expression:

$$\text{Log(Reduction)} = \log_{10} \frac{C_0}{C(t)} = \log_{10} C_0 - \log_{10} C(t) \quad (1)$$

where: C_0 [cfu/mL] is the initial bacteria concentration; C_t [cfu/mL] is the total suspended bacterial concentration detected at time t.

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

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

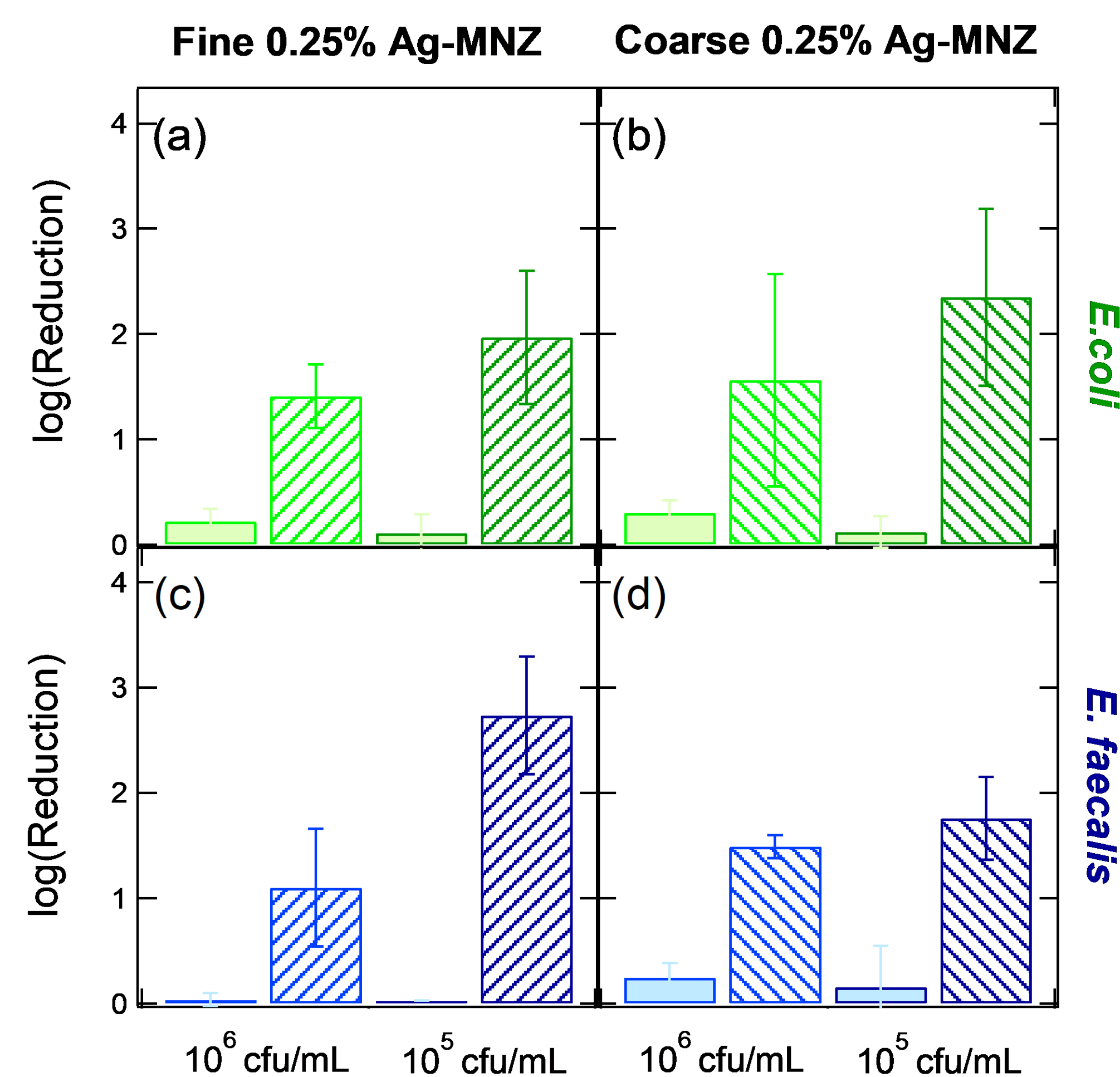


Figure 2: Calculated log-reduced values of *E. coli* (a,b) and *E. faecalis* (c,d) in controls (filled columns) and interacting with fine (0-1 mm) 0.25% Ag-MNZ (a,c) and coarse (1-3mm) 0.25% Ag-MNZ (b,d) for bacterial initial concentrations: 10^6 and 10^5 cfu/mL (cross-shaded columns) within 1h of experiment.

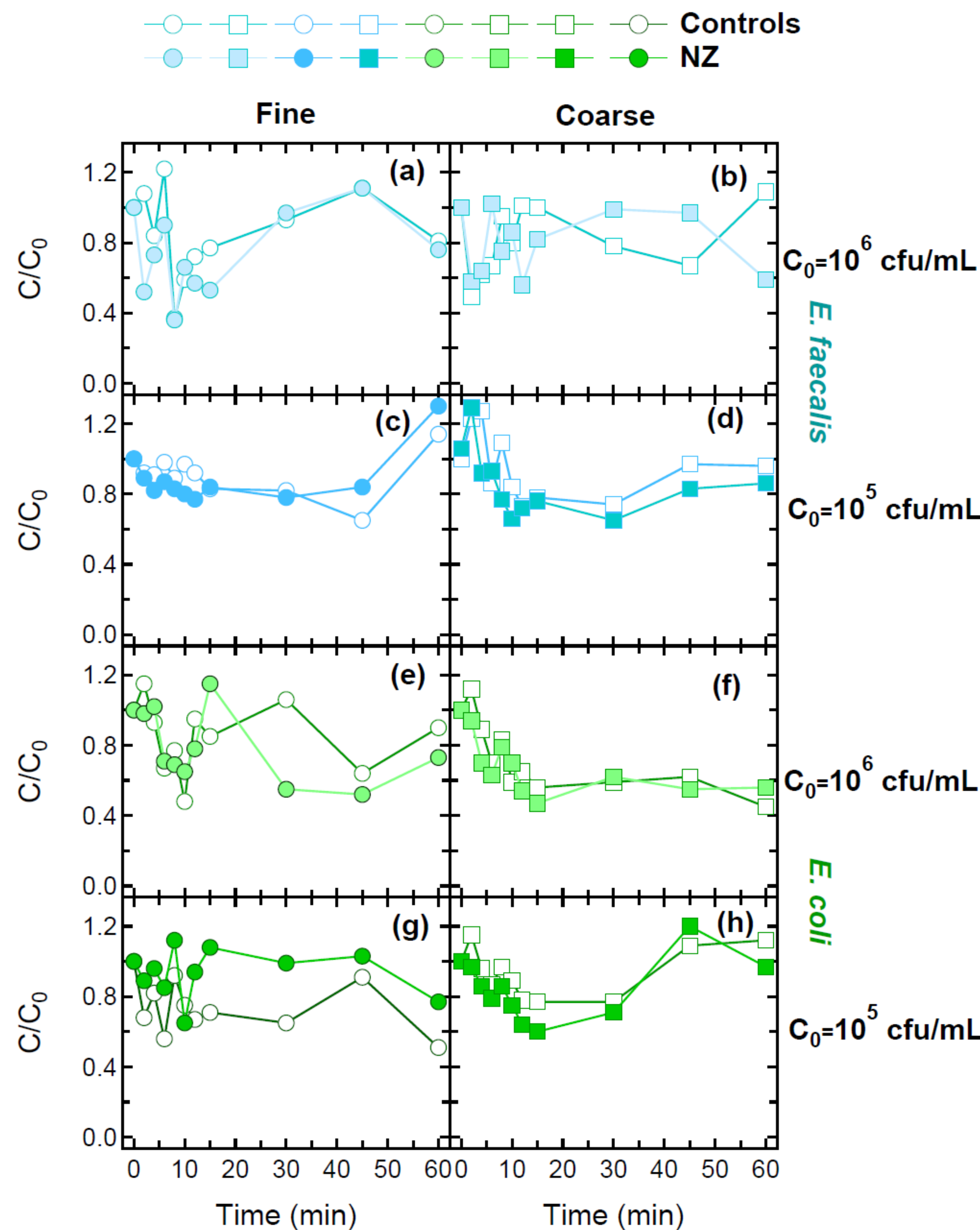


Figure 3: Experimental data of *E. faecalis* (a-d) and *E. coli* (e-h) removal by fine (0-1 mm) NZ (a-g) and coarse (1-3mm) NZ (b-h) in the absence (empty symbols) and the presence (solid symbols) of zeolites. Bacterial initial concentrations: 10^5 and 10^6 cfu/mL

Table 2: Calculated log and percent reduction values of *E. coli* with the period of 1, 6 and 24 hours under the experimental conditions

Experimental conditions/ Initial bacteria concentration (cfu/mL)	<i>E. coli</i> -Fine NZ (0-1 mm)				<i>E. coli</i> -Coarse NZ (1-3 mm)			
	Control		Reactor		Control		Reactor	
	Log(Reduction)	P (%)	Log(Reduction)	P (%)	Log(Reduction)	P (%)	Log(Reduction)	P (%)
1 hour								
10^6	0.19	36.13	0.29	48.39	0.16	30.17	0.14	28.21
10^5	0.29	48.65	0.11	22.52	0.01	3	0.02	3.41
10^4	0.16	30.63	0.28	47.64	0.07	15.38	0.16	30.77
10^3	0.67	78.71	0.58	73.95	0.02	4.35	0.1	20.35
6 hours								
10^6	0.25	43.87	0.39	59.68	0.14	28.21	0.17	32.05
10^5	0.3	49.55	0.18	33.33	0.18	34.66	0.16	31.25
10^4	0.56	72.64	0.44	63.3	0.17	32.69	0.25	44.23
10^3	0.96	89.08	0.63	76.47	0.26	45.65	0.16	30.43
24 hours								
10^6	0.29	48.71	0.24	42.9	0.21	38.46	0.2	37.18
10^5	0.87	86.49	0.63	76.58	0.9	87.5	0.6	75
10^4	0.7	80.04	0.56	72.64	0.24	42.31	0.3	50
10^3	0.73	81.51	0.66	78.15	0.6	75	0.55	71.74

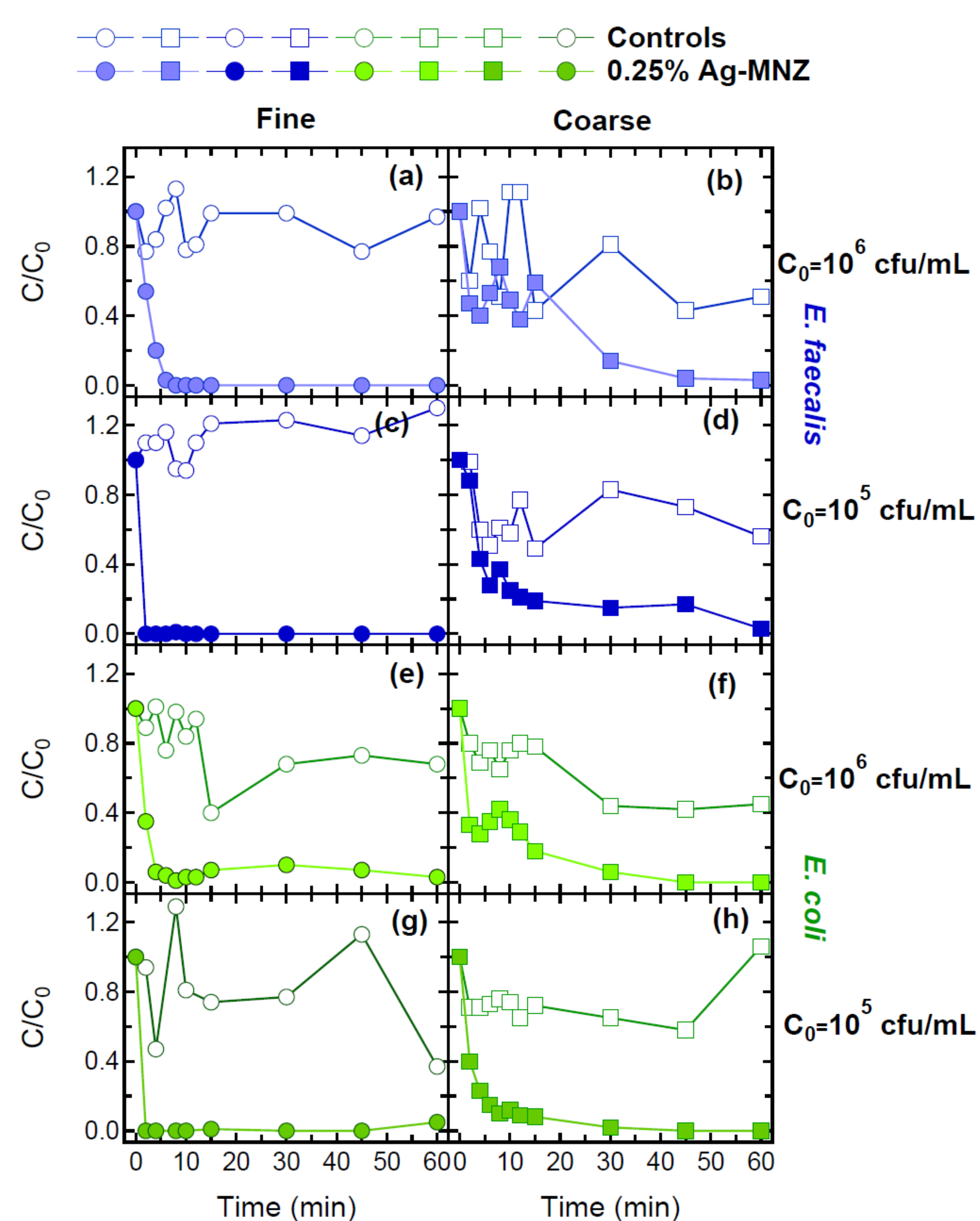


Figure 4: Experimental data of *E. faecalis* (a-d) and *E. coli* (e-h) removal by fine (0-1 mm) Ag-MNZ (a-g) and coarse (1-3mm) Ag-MNZ (b-h) in the absence (empty symbols) and the presence (solid symbols) of zeolites. Bacterial initial concentrations: 10^5 and 10^6 cfu/mL

Conclusions

- In the control tubes within 1 h of experiment no significant changes in the bacterial numbers were observed when compared to the initial concentrations.
- After 24 h of experiment, *E. coli* showed similar reduction in the bacterial numbers in the controls and reactors with NZ. However higher percent reduction observed for fine than coarse NZ.
- The Ag-MNZ resulted in much higher reduction of the both bacterial numbers when compared to the NZ and controls.
- Earlier bacterial removal was observed in the case of fine than coarse Ag-MNZ for both bacteria.
- The Ag-MNZ reduced both bacteria from 1.1 to 2.74 logs after 1 hour.
- In the most cases examined, the reduction in the bacterial numbers increased with decrease of initial bacterial concentrations.

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