

Removal of Bacteria from Wells Water in Erbil City using Natural Zeolite

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Abstract— Although a body of studies is available on the classical methods used for preparing drinking water free of bacterial and other microorganism contamination, a spare number of such studies were carried out, on testing of the natural volcanic mineral, zeolite. Therefore, this study intended to examine the effect of a promising natural zeolite, clinoptilolite, on the removal of bacteria from water samples taken from two wells present in two different regions of the province Erbil/Iraq, Nowruz and Sebardan. Five water samples of each region were tested before and after treatment with the clinoptilolite to isolate and enumerate viable microorganisms for total bacterial count (TBC). A batch system technique for automating and processing the samples from the wells was adopted. The first experiment treated the five samples separately with 1, 2, 3, 4, and 5 grams of clinoptilolite for 1 hour. In the second experiment, all five samples were treated with 1 gram of zeolite but exposed to the treatment for 1, 1.5, 2, 2.5, or 3 hours. The TBC of the water obtained from the two regions (Nowruz and Sebardan) before treatment with zeolite were 19.4 and 8.6 colony-forming units (CFU) respectively, whereas the results after treatment with zeolite were, 4.8 CFU and 2.00 CFU, respectively. The results also showed that the most effective treatment caused the highest reduction in the CFU number which was with 1 gram of zeolite for three hours of contact time. The result indicated a significant effect of natural zeolite on removing the bacterial contamination from well water. Also, it showed that the water of the Nowruz region is more contaminated than that of the Sebardan region.

Index Terms— Natural zeolite, removing bacteria, Total Bacteria Count.

I. INTRODUCTION

The name zeolites comes from the Greek words “zeo” which means boiling and “lithos” which means stone. It means stones evolved water vapor when gently heated. Natural or synthetic zeolites are inorganic crystalline solids with small pores in the range of 1-20 angstrom diameter running throughout the solid [1].

Zeolites are hydrated aluminosilicates of alkaline and alkaline-earth metals with fully cross-linked open framework structures comprising corner-sharing SiO₄ and AlO₄ tetrahedral [2].

The general formula of zeolites can be written as (A^{+z})_{x/z} (Al³⁺)_y (Si)_x O_{2(x+y)} · nH₂O where A represents extra framework cations, z is the charge on the extra framework cations, n is the number of moles of molecular water. X and y are the stoichiometric coefficients for Al³⁺ and Si⁴⁺ in tetrahedral sites, respectively [3]. The oxide expression of the chemical formula of zeolites is M_{2/n} O, Al₂O₃, xSiO₂, yH₂O [4], [5], where M represents the exchangeable cation of valence n. M is generally a group I (Na, K, Li) or group II (Ca, Mg, Ba, Sr) ion, although

other metal, non-metal, and organic cations may also balance the negative charge created by the presence of Al in the structure.

There are about 40 naturally occurring zeolites known and about another 150 or so that have been synthesized. Naturally occurring zeolites are rarely pure and are contaminated to varying degrees by other minerals, metals, quartz, or other zeolites. Both natural and synthetic zeolites are used commercially because of their unique properties like a rigid, 3-dimensional crystalline structure consisting of a network of interconnected tunnels and cages. Zeolites belong to the molecular sieves family of microporous solids. These materials can sort molecules based on size due to the regular pore structure of zeolites. In addition, zeolites can contribute to a cleaner and safer environment. For example, zeolites are used in the petroleum refining and hydrocarbon industry, and the chemical processes involved became more efficient by using zeolites as catalysts thus saving energy and indirectly reducing pollution. Furthermore, zeolites can also act as solid acids which could reduce the need for corrosive liquid acids, and as redox catalysts and sorbents where they can remove atmospheric pollutants such as engine exhaust gases and ozone-depleting chlorofluorocarbons [6]. Zeolite can separate harmful organics [7] and remove heavy metal ions including those produced by nuclear fission of water [8], [9]. Zeolites have many unique properties such as porous structure, regular shape of the pores in the zeolites framework, and high acidity of the rigid framework when the cations are protons. These unique properties in zeolites have found their uses in many applications, particularly as ion exchangers, absorbents, molecular sieves, and catalyst. The production of zeolites has increased in the last thirty years showing the big growth of zeolite industries especially in fine chemical industries, animal food, waste treatment, desiccant, water purification, building agents in detergents that soften the water, catalyst for chemical reactions and gas absorbent markets [10]. In addition, zeolites, which are exchanged with the nutrient ions, ammonium and potassium, can be added to soils as ionic-type fertilizers. Zeolites are added to the diets of pigs, chickens, and ruminants to increase their body weights. Zeolites can be used as electrical conductors where the mobile cations that are located in the zeolite channels impart ionic electrical conductivity [10], [11]. Both the pure and the impure zeolitic materials have high potential in the application of waste-water and flue gas-cleaning technologies [12].

Growing population, shrinking water resources, and increasing pollution from industrial and household effluent activities have rendered polluted water resources even more critical. Water purification of wastewater from industries and households has gained favorable attention for many years [13, 14]. Wastewater

treatment aims to extract pollutants from water resources. Water pollutants, such as soluble and insoluble heavy metals and some bacteria may be highly toxic to humans and the aquatic environment [15]. Methods currently available to limit pollutants and dissolved toxins include ultrafiltration [16], advanced separation of oil–water [17], use of hydrocyclones [18], chemical clarification [19], and gas flotation [20].

There is very little use of zeolite to remove bacteria from contaminated water in the literature review. In this study, the effect of zeolite on the removal of bacteria from contaminated water was investigated.

II. MATERIALS AND METHODS

The natural zeolite, clinoptilolite which is used in this study was of Turkish origin and obtained from the local market. The chemical composition of the clinoptilite was identified by X-ray fluorescence analysis (XRF), as shown in Table (1).

A. Sample collection

The tested water that was used in this investigation was collected from two wells located in the Nowruz and Sebardan regions, of Erbil province/Iraq. The samples were collected from each well according to the standards and guidelines. Sterilized and free of contamination dry glass bottles were used and the samples were manually collected from one drill hole of each well.

B. Removal of bacteria using the zeolites in a batch system

To determine the sterilizing effect of the natural zeolite on the water samples in this investigation, the experiment was carried out soon after the samples collection, The batch system technique was used to test the efficiency of zeolite in removing bacteria.

In this study, the effect of two factors on the removal of bacteria from water samples was examined. The two factors were the amount of zeolite and the contact time of the zeolite with the samples. The samples were subjected to a bioburden test before and after contacting the samples with the zeolite.

To determine the effect of zeolite on bacteria removal, different amounts of zeolite, 1, 2, 3, 4, and 5 grams were added to five samples of 10 ml in an Erlenmeyer flask. The five samples of suspended solutions were mixed with a laboratory magnetic stirrer and kept in contact with zeolite for 1 hour. In the second part, 1 gram of zeolite was added to another five samples but for a period time of 1, 1.5, 2, 2.5, and 3 hours. After the period of the contact times, the suspended solutions were filtered and the filtrates were examined for total bacterial count.

C. Total Bacteria Count

All glassware and tools used for the bacterial test were sterilized with alcohol. A Bunsen burner was also installed for added safety. 24 hours before conducting the experimental work, nutrient agar was prepared to support the growth of a wide range of nonfibrous organisms. Before treating the samples with zeolite, the TBC test was performed by placing 10 ml of water sample in a petri dish and 10 ml of nutrient agar which was added. The Petri dish was then placed in an autoclave for

48 hours. Whereas, after treating the samples with zeolite, the TBC test was performed when 10 ml of each sample was added to a certain amount of zeolite in an Erlenmeyer flask.

The resulting suspended solutions were stirred for some time and then filtered. The filtrate was placed in a petri dish, and 10 ml of nutrient agar was added to the filtrate. The Petri dish with the filtrate and nutrient agar was placed in the autoclave for 48 hours. At the end of the time, the petri dish was divided into 4 triangles, and the only triangle was written, how many bacteria are in a triangle and equal to 4. Number of bacteria in a triangle * 4 triangles petri dish * 10.

III. RESULTS AND DISCUSSION

Zeolite is a non-toxic, three-dimensionally porous, crystalline, hydrated aluminosilicate with natural adsorption and ion exchange properties that removes harmful microbes and dispersed insoluble and soluble toxins from drinking water. The results of the XRF (X-ray fluorescence) test for analyzing the chemical structure of natural zeolite are shown in Table (1). The higher percentage concentrations of oxides in the natural zeolite were 68.518 and 12.323, which belong to silica and alumina, respectively. i.e., the fully crosslinked open framework structures of zeolite, which consist of corner-sharing SiO₄ and AlO₄, tetrahedral of silicon oxide, and aluminum oxide. This structure allows the formation of small pores in the range of 1-20 angstrom diameter running throughout the solid [1].

Table (1) XRF test of the chemical structure of natural zeolite Clinoptilolite:

Compound/Element	Unit	AB1
SiO ₂	%	68.518
Al ₂ O ₃	%	12.323
Fe ₂ O ₃	%	2.393
Ca O	%	3.144
MgO	%	1.51
Na ₂ O	%	0.602
K ₂ O	%	3.058
TiO ₂	%	0.108
Mn O	%	0.068
P ₂ O ₅	%	0.016
Loi (Loss on Ignition)	%	8.18
S	ppm	91
Cl	ppm	774
Sr	ppm	1992
V	ppm	27
Zn	ppm	14
Zr	ppm	20
Ce	ppm	29
La	Ppm	42
Pb	ppm	N

Before and after contact with zeolite, the TBC of water samples from the Nawrouz district were determined and are given in Table (2). In general, the results show a decrease in TBC levels after exposure to zeolite, reflecting zeolite's ability to remove bacteria from contaminated wells water. The values of TBC

before and after exposure to zeolite were 19.4 CFU and 5.54 CFU, respectively. 5.54 CFU represents the lowest value of TBC, which belongs to the run where the amount of zeolite was 4 g and a fixed contact time of 1 hour. This means the higher bacteria removal when TBC was changed from 19.4 to 5.54 CFU. This is due to the increase in adhesion of bacteria on the surface of zeolite. The decrease in TBC appears to be observed when comparing the values represented by the bars in Figure (1).

Table (2) Total bacterial count before and after contact of 10 ml of the samples obtained from (Newroz) with different amounts of zeolite for 1 hour.

Amount of zeolite (g)	Total bacteria count (CFU) before contact	Total bacteria count (CFU) after contact	The removal percentage
1	19.4	6.2	68
2	19.4	5.9	69.58
3	19.4	7.00	63.9
4	19.4	5.54	71.4
5	19.4	5.7	70.6

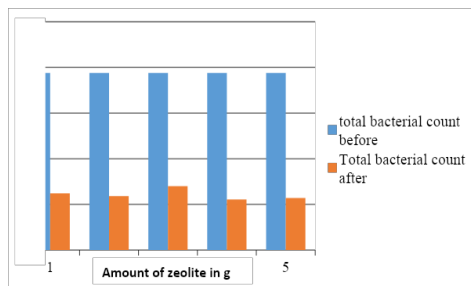


Figure (1) shows the TBC of samples obtained from (Nawrouz District) before and after exposure to various amounts of zeolite.

On the other hand, the results of the TBC values in the case of a different contact time and an amount of zeolite fixed to 1 g are shown in Table (3). Results show a decrease in TBC from 19.4 CFU to 4.8 CFU. Comparing the results in Table (2) with the results in Table (3) shows that the contact time is more effective in removing bacteria than the amount of zeolite. As contact time is increased, TBC values appear to decrease to 4.8 CFU consistently. In contrast, on increasing the zeolite amount, the TBC values shown in Table (2) were randomly changed to reach 5.54 CFU. This difference was because it takes more time for bacteria to stick to the surface of zeolite.

A consistent decrease in TBC appears to be observed when comparing the values represented by the bars in Figure (2).

Table (3) Total bacterial count before and after contact of 10 ml of the samples obtained from (Nawrouz district) with 1 g zeolite for different contact times.

Time of contact (hour)	Total bacterial count (CFU) before contact	Total bacterial count (CFU) after contact	The removal percentage
1	19.4	6.2	68
1.5	19.4	6.00	69
2	19.4	5.6	71.1
2.5	19.4	5.3	72.6
3	19.4	4.8	75.2

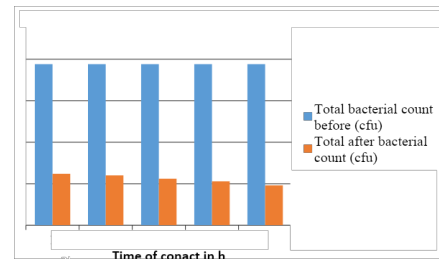


Figure (2) shows the TBC of 10 ml samples obtained from (Newroz) before and after contact with 1 g zeolite at different times.

The TBC of water samples from the Seberdan district before and after exposure to zeolite were counted and reported in Tables (4 and 5). The same impression was made in the case of the Newroz district. Results show a decrease in TBC levels after exposure to zeolite, reflecting zeolite's ability to remove bacteria from contaminated wells water. As shown in Table (4), The same random pathway of decrease in TBC values of the Seberdan district was observed compared with the results of the Newroz district. The TBC values after contact of 10 ml of the samples obtained from the Seberdan district with different amounts of zeolite for 1 hour were observed when comparing the values represented by the bars in Figure (3). It indicates how effective the contact time is in removing bacteria compared to the amount of zeolite. As contact time is increased, TBC levels appear to decrease to 2 CFU consistently.

By increasing the amount of zeolite, the TBC values were randomized to reach the lowest value of 2.1 CFU. This difference is because bacteria need more time to attach to the surface of the zeolite.

Table (4) Total bacterial count before and after contact of 10 ml of the samples obtained from (Seberdan) with different amounts of zeolite for 1 hour.

Amount of zeolite (g)	Total bacterial count (CFU) before contact	Total bacterial count (CFU) after contact	The removal percentage
1	8.6	3.2	62.7
2	8.6	3.00	65.1
3	8.6	2.3	73.2
4	8.6	2.1	75.5
5	8.6	3.00	65.1

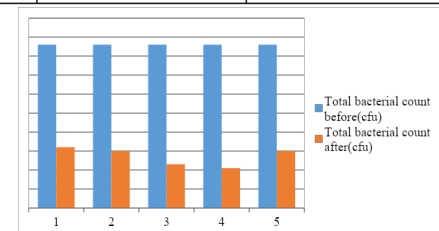


Figure (3) shows the TBC of samples obtained from (the Seberdan District) before and after contact with different amounts of zeolite.

As shown in Table (5), the results after contact with 1 g of zeolite at different periods show a consistent decrease in CFU values. Also, the highest removal percentage among all TBC values was 76.7. The set of conditions leading to the highest removal percentage were 3 h contact time and 1 g amount of zeolite. The results show high well water contamination from Newroz compared to the Seberdan district samples. This is due to the exposure of the well hole to the outside environment and the large number of cattle in the area. The consistent decrease in CFU values is shown in Figure (4).

Table (5) Total germ count before and after contact of 10 ml of the samples obtained from (Seberdan) with 1 g zeolite for different contact times.

Time of contact (hour)	Total bacterial count (CFU) before contact	Total bacterial count (CFU) after contact	The removal percentage
1	8.6	3.2	62.7
1.5	8.6	2.8	67.4
2	8.6	2.8	67.4
2.5	8.6	2.3	73.2
3	8.6	2.00	76.7

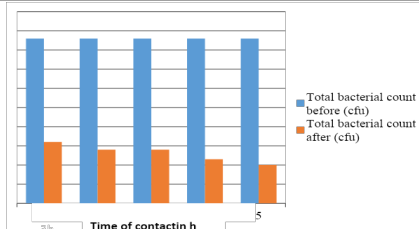


Figure (4) shows the TBC of 10 ml samples obtained from (Seberdan) before and after contact with 1 g zeolite for different contact times.

CONCLUSION

The natural zeolite type clinoptilolite has a good ability to remove bacteria from the water. Removal occurs when the bacteria adhere to the surface of the zeolite. The zeolite surface consists of many pores, cavities, and channels, which are considered to be a good platform for bacteria to attach. In this work, the removal of bacteria from well water and the effect of the two factors (amount of natural zeolite and time of contact with zeolite) were studied. The results showed that with a contact time of 3 hours and a zeolite amount of 1 g, 76.7% of the bacteria were removed. The result also shows the higher effect of contact time with zeolite on the removal process compared to the amount of zeolite.

REFERENCES

- [1] Breck. D. W., Zeolite and molecular sieve, structure, chemistry and use. New York: John Wiley and Sons. 1974.
- [2] Lesley E.Smart and Elaine A. Moore, Solid state chemistry. Third Edition ed. London: Taylor & Francis Group. 2005.
- [3] Philip S. Neuhoff and Laura S. Ruhl, "Mechanisms and geochemical significance of si-al substitution in zeolite solid solutions", Chemical Geology, p. 373-387. 2006. 225.
- [4] M. M. Rahmana, N. Hasnidab, and W.B.W. Nikb, "Preparation of zeolite y using local raw material rice husk as a silica source". J. Sci. Res., p. 285-291. 2009. 1.
- [5] WANG Yanxin, et al., "Synthesis of zeolites using fly ash and their application in removing heavy metals from waters". SCIENCE IN CHINA (Series D), p. 967-976. 2003. 46.
- [6] Koichi Mizukamia, et al., "Atomistic mechanism of the adsorption of CFCs in zeolite as investigated by Monte Carlo simulation". Studies in Surface Science and Catalysis, p. 1811-1818. 1997. 105.
- [7] Hooykaas and Carel W. J., "Method for capturing ecologically harmful substances from material polluted with such substances in reverse osmosis today", Pelt & Hooykaas B.V. (NL) NL. 1995.
- [8] E. Álvarez-Ayuso, A. García-Sánchez, and X. Querol, "Purification of metal electroplating waste waters using zeolites", Water Research, p. 4855-4862. 2003. 37.

- [9] Wei Qiu and Ying Zheng, "Removal of lead, copper, nickel, cobalt, and zinc from water by a cancrinite-type zeolite synthesized from fly ash". Chemical Engineering Journal, p. 483-488. 2009. 145.
- [10] Dyer A., An Introduction to Zeolite Molecular Sieves. 1988, Brisbane: John Wiley and Sons.
- [11] Szostak R., Molecular Sieves Principles of Synthesis and Identification. 1989, New York: Van Nostrand Reinhold.
- [12] Natalia Moreno, et al., "Pure zeolites synthesis from silica extracted from coal fly ashes", J. of Chem. Technol. and Biotechnol, p. 274-279. 2002. 77.
- [13] Crini, G., Lichtfouse, E., Wilson, L.D., and Morin, N., "Conventional and non-conventional adsorbents for wastewater treatment", Environmental Chemistry Letters, pp. 195-213, 2019. 17 (1), <https://doi.org/10.1007/s10311-018-0786-8>
- [14] Calabrò, P.S., Bilardi, S., and Moraci, N., "Advancements in the use of filtration materials for removing heavy metals from multicontaminated solutions", Current Opinion in Environmental Science & Health, pp. 100241, 2021. 20. <https://doi.org/10.1016/j.coesh.2021.100241>
- [15] Chen, X., Yu, L., Zou, S., Xiao, L., and Fan, J., "Zeolite Cotton in Tube: A Simple Robust Household Water Treatment Filter for Heavy Metal Removal", Scientific Reports, 10, pp. 4719, 2020. <https://doi.org/10.1038/s41598-020-61776-8>
- [16] Hembach, N., Alexander, J., Hiller, C., Wieland, A., and Schwartz, T., "Dissemination prevention of antibiotic-resistant and facultative pathogenic bacteria by ultrafiltration and ozone treatment at an urban wastewater treatment plant", Scientific Reports, 9(1), pp. 12843, 2019. <https://doi.org/10.1038/s41598-019-49263-1>
- [17] Gupta, R.K., Dunderdale, G.J., England, M.W., and Hozumi, A., "Oil/water separation techniques: a review of recent progress and future directions", Journal of Materials Chemistry A, pp. 16025-16058, 2017. 5(31). <https://doi.org/10.1039/C7TA02070H>
- [18] Pećarević, M., Mikuš, J., Prusina, I., Juretić, H., Bratoš Cetinić, A., and Brailo, M., "The new role of hydrocyclone in ballast water treatment". Journal of Cleaner Production, pp. 339-346, 2018. 188. <https://doi.org/10.1016/j.jclepro.2018.03.299>
- [19] Pérez-Calderón, J., Santos, M. V, and Zaritzky, N., "Optimal clarification of emulsified oily wastewater using a surfactant/chitosan biopolymer". Journal of Environmental Chemical Engineering, pp. 3808-3818, 2018. 6(4). <https://doi.org/10.1038/s41598-019-49263-1>
- [20] Kyzas, G.Z. and Matis, K.A., "Flotation in water and wastewater treatment. Processes", p.116, 2018. 6(8). <https://doi.org/10.3390/pr6080116>