

The Use of Surfactant-Modified Natural Zeolite for Removal of Bacteria

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Abstract: Improving the efficiency of Indonesia's natural zeolites can be done through a modification process. The expected results of the natural zeolite modification can be applied to several remedies to the interests of the environment to control the population of pathogenic microorganisms that tend to have a negative charge. Natural zeolite is aluminosilicate compounds with porous structure and a negative charge on the surface. Zeolite surface negative charge is neutralized by cations that can be exchanged with other cations, including cationic surfactant. Cationic surfactants such as HDTMA⁺ (Hexadecyltrimethylammonium) has a large molecular size that can only be exchanged with cations in the external surface of natural zeolite. Zeolite surface modification was carried out through the activation phase with the addition of 1% HF solution and NH₄NO₃ 2M, followed by exchange of cations through the addition of HDTMA⁺. Zeolite characterization was performed using Infrared Spectroscopy and Scanning Electron Microscopy (SEM). Result of Infrared spectroscopy analysis shows the peak of 2850.68 cm⁻¹ and 2919.63 cm⁻¹. Analysis using SEM showed the surface had been coated with HDTMA⁺ surfactant which indicated modifications occurred on the surface of zeolite. The results of bacteria adsorption test using natural zeolite and modified zeolite, they could adsorb consecutively 5.6 and 100% of the bacteria *Staphylococcus aureus* and 7.2 and 99% of the bacteria *Escherichia coli* bacteria with the initial concentration of *Staphylococcus aureus* 1.8 x 10¹² CFU/mL and the bacteria *Escherichia coli* 6.9 x 10¹¹ CFU/mL at doses of 3 g of zeolite and with contact time of 4 h.

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I. Introduction

The zeolite mineral is composed of hydrated aluminosilicate crystals containing an alkali metal or alkaline earth metal cation in its three-dimensional framework. These metal ions can be replaced by other cations without damaging the structure of the zeolite [1]. Balancing cations load on the surface of the zeolite; such as Na⁺, K⁺, Ca²⁺ and Mg²⁺ may also be replaced by quaternary ammonium molecules having a higher molecular weight, such as hexadecyltrimethylammonium HDTMA⁺, C₁₆H₃₃N(CH₃)³⁺ [2]. The exchange between cations and HDTMA⁺ molecules takes place on the outer surface of the zeolites because the size of the HDTMA⁺ molecules is too large and can not penetrate the internal pore system of angstrom-sized zeolite [2,4,5]. Modification using cationic surfactants such as HDTMA⁺ is aimed to modify the chemical surface of the initially negative to positive natural zeolites [4,5]. The positively charged surfaces can be used to adsorb anions such as chromates, nitrates and other negatively charged species, including pathogenic micro-organisms [2,3]. During the last twenty years, at least 342 diseases have been caused by groundwater contamination, 30% of which is caused by contamination of pathogenic microorganisms [6]. To control microorganisms by modifying natural zeolites using cationic surfactants, several studies have been carried out, in particular on the adsorption of MS2 viruses and *Escherichia coli* bacteria, microorganisms of *Giardia lamblia*, intestinal *Giardia* and organisms of the *Saccharomyces cerevisiae* model using natural zeolites [7,10]. According to [6], pathogenic bacteria and microorganisms that live in the subsoil generally have a negative charge. Therefore, by modifying the natural zeolites using cationic surfactants, it is expected that pathogenic bacteria can be adsorbed on the surface of the naturally-modified, positively charged zeolite [8]. The natural zeolite from Bayat, Indonesia is included in the mordenite type associated with clinoptilolite and quartz. In this research, a natural surface modification of the zeolite was performed using a cationic surfactant HDTMA⁺ and determination of the optimal concentration of HDTMA⁺ in the cation exchange process. The adsorption tests for pathogenic bacteria carried out on modified zeolite were *Escherichia coli* and *Staphylococcus aureus*. adsorption activities of *Escherichia coli* and *Staphylococcus aureus* on modified zeolites were evaluated.

II. Material And Methods

Materials:

A sample of natural zeolite was collected from an open clay deposit in Bayat, Central Java, Indonesia, aquadest; HF (Merck); HDTMA-Cl (Merck); NH_4NO_3 (Merck); 2-propanol (Merck); nutrient medium agar, nutrient broth medium, phosphate buffer, culture of *Escherichia coli* and *Staphylococcus aureus*.

Methods:

Activated Natural Zeolite:

A sample of natural zeolite was collected from an open clay deposit in Bayat, Central, Java, Indonesia. The natural zeolite was sieved (-60/+100 mesh). Then, the natural zeolite was washed with distilled water and immersed in a 1% HF solution for 10 min. Then, the natural zeolite was heated in an oven at 120 °C for 4 h. The dried natural zeolite was soaked in 2M NH_4NO_3 for 4 h and then washed with distilled water to neutral pH. Finally it was calcinated at 250°C for 4 h. The activated zeolite then called as ZA.

Modified of Natural Zeolite:

Modifications were made to the active zeolite, the column filled with 5 g of zeolite. A solution of HDTMA⁺ 0.20 M was then added to the zeolite which was then called ZMS. Then, each sample was added with a solution of HDTMA⁺ of 25 ml with a flow rate of 6 seconds/drops. Then the filtrate is collected and the residue dried. The results of the natural modification of the zeolite were then analyzed by FTIR and SEM.

Adsorption activity:

Adsorption tests on the dichromate ions were carried out on natural and modified zeolites. A solution of dichromate at a flow rate of 1 drop/6 seconds was then added to the packed column of 2 gram zeolite. Finally, the filtrate of dichromate ion concentration was analyzed by atomic absorption spectroscopy (AAS). The bacteria *Escherichia coli* and *Staphylococcus aureus* were cultured on Nutrient Broth medium (Merck) with stirring at 120 rpm for 24 h at room temperature. Then the number of bacterial cells was counted by the Total Plate Count (TPC) method. The growth-derived bacterial suspension was added into the column consisted of 3 g of natural zeolite or modified at pH 7. After 4 h, the bacterial suspension was flowed at a rate of 1 drop/ 6 seconds. Next, the number of unadsorbed bacterial cells by the zeolites was counted by TPC method. Optimization was performed for the culture pH, the zeolite dose and the contact time between the bacteria and the zeolite. To optimize the pH, the zeolite dose (3 g/25 mL) was performed on bacterial cells and then added to a phosphate buffer having a pH of 6.0; 7.0; and 8.0. For the optimization of the dose, the zeolites were varied using 1, 3 and 5 g / 25 mL, while the contact times of 4; 8; and 12 h for the bacteria containing zeolites at pH 7 were performed.

III. Result and Discussion

The surface of the zeolite has a negative charge which is stabilized by a balancing cation. The counterweight cation can be replaced or exchanged in the presence of cationic surfactants such as (HDTMA⁺) through two steps, namely cation exchange mechanisms and hydrophobic interactions. This modification process is intended to increase the ability of the zeolite to absorb anions in solution. The exchange of natural zeolite cations with surfactant molecules can be performed using a column system. The use of columns should allow a more complete contact between the surface of the zeolite and the molecule (HDTMA⁺), so that the cation exchange between the surfactant molecules (HDTMA⁺) and the equilibration cations at the surface of the zeolite can occur. The mechanism is through surfactant cation exchange to form a monolayer between the zeolite and the surfactant on the surface of the zeolite. As the surfactant concentrations increase, there will be an interaction between the ends of the surfactant which hydrophobically binds with the other hydrophobic ends to form bilayer. Solution (HDTMA⁺) was used to soak the zeolite up to 25 mL with a flow rate of 6 drops/second. The immersion of the zeolite and the flow rate of the solution (HDTMA⁺) always make it possible to eliminate the filtrate from the solution (HDTMA⁺) of the column. Although the drainage is too fast, the cation exchange between the zeolite and the surfactant solution is less than optimal. Analysis of infrared spectra Infrared spectroscopy is one of the methods used to determine the structure of the zeolite. In general, the wave number range of 300-300 cm^{-1} as tetrahedral bonds, namely $(\text{SiO}_4)^{4-}$ and $(\text{AlO}_4)^{5-}$ [9]. The 420-500 cm^{-1} spectrum corresponds to the flexion of Si-O or Al-O, whereas in wavenumbers of 750 to 820 cm^{-1} , symmetrical stretching and asymmetric stretching occur in the wave numbers of 950-1250 cm^{-1} [10]. FTIR spectral data show Si-O or Al-O bending in natural zeolites observed at wave number 490.00 cm^{-1} . In zeolite modified with (HDTMA⁺), a band appears around 2800-2900 cm^{-1} [11].

In the HDTMA zeolite, a band of 1400.81 cm^{-1} appears, which corresponds to the flexural vibration of N-H. The 2800-3000 cm^{-1} band shows symmetric and asymmetric CH_2 stretching of the amine [11]. In natural zeolites modified with HDTMA⁺ appear especially with the band appearances of 2850.68 and 2919.63 cm^{-1} . According to [11], symmetrical and asymmetric CH_2 frequencies and bandwidths for amines depend on the

concentration of amine.

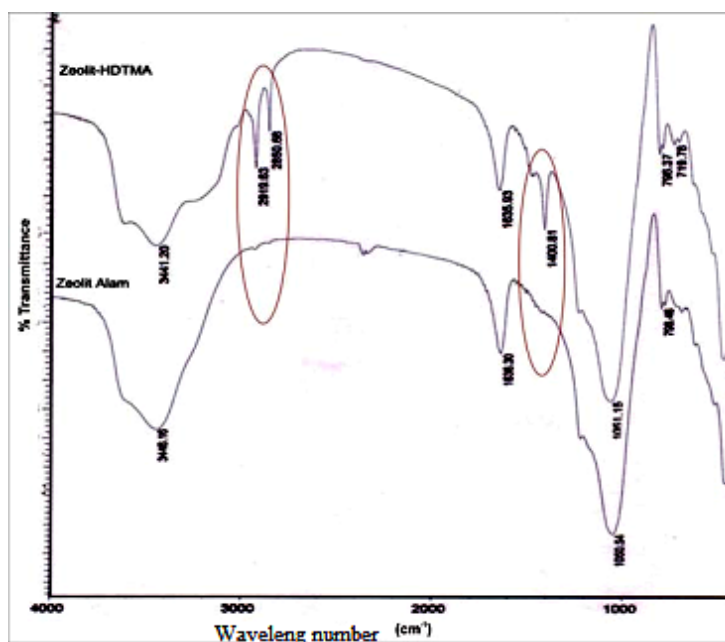


Fig 1. Infrared spectra of natural zeolite and HDTMA-modified zeolite

Analysis of surface morphology using a scanning electron microscope (SEM) instrument was conducted to support the FTIR analysis according to which a bilayer layer had been formed on the surface of a modified zeolite by surfactant HDTMA⁺. A comparison of the surface morphology of the SEM imaging results was performed on natural zeolites and modified zeolites. The results obtained from the morphological surface analysis of the SEM imaging shows a clear difference between the surface of the natural zeolite and the surface of the HDTMA⁺ modified zeolite. The difference in surface morphology of the SEM imaging results shows that a modified zeolite surface had been formed, which enhances the appearance of the IR spectra at 2850.68 and 2919.63 cm⁻¹, while on the surface, natural zeolites appear to be coarser and not covered with molecules.

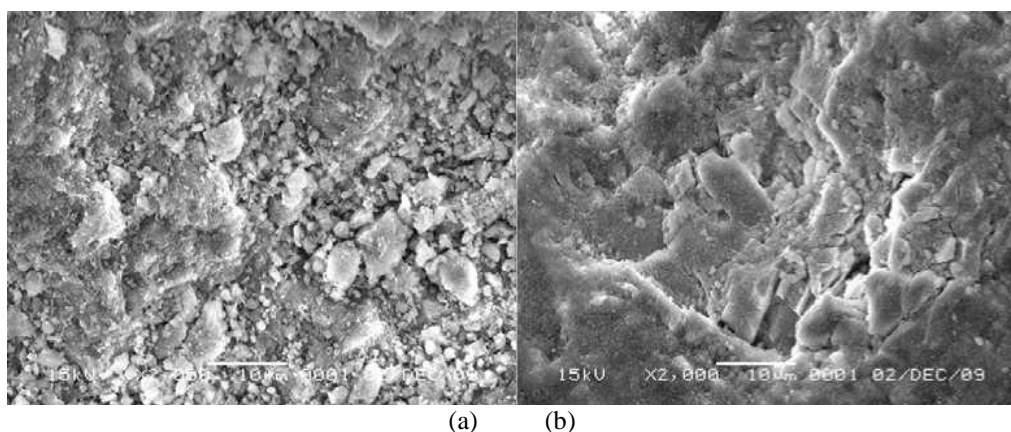


Fig 2. SEM image (a) natural zeolite and (b) HDTMA- modified zeolite

The adsorption mechanism of surfactants on the zeolite surface takes place through the ion exchange process and hydrophobic interactions, starting with the cation exchange process on the surface of the zeolite with cationic surfactant molecules to form a single layer (monolayer) between the surfaces of the zeolite and the surfactant. The increase in the concentration of surfactants causes interactions between the hydrophobic tail of the surfactant, so that there is a bond with the other hydrophobic tail which then forms a bilayer. The formation of a double layer causes a positive charge modification of the zeolite surface, so that the anions can be adsorbed on the surface of the zeolite [2].

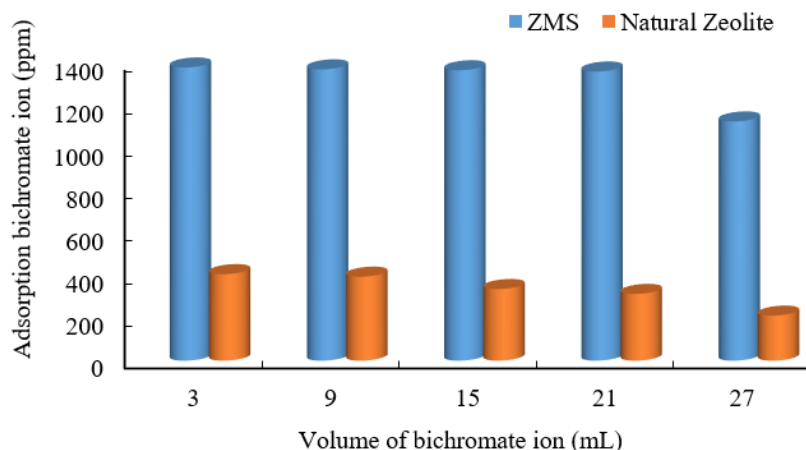


Fig 3. Adsorption activity of bichromate ion on natural zeolite and HDTMA⁺-modified zeolite (ZMS)

From Figure 3, it appears that natural zeolites are also able to adsorb the dichromate ions. This adsorption process occurs because the dichromate ions are trapped in the natural zeolite pore system, and not because of the charge interactions. While in the modified zeolite (ZMS), the adsorption process occurs due to the electrostatic interaction between the positive charge of the modified zeolite and the negative charge of the dichromate ion. The tendency of this property shows that the positive surface area of the zeolite has been saturated due to interactions with the dichromate ion, so that the surface of the zeolite is unable anymore to adsorb the dichromate ion. The results of the atomic absorption spectroscopy analysis yielded the maximum concentration of dichromate absorbed for 27 mL of dichromate solution were 212 ppm for natural zeolites and 1125 ppm for modified zeolites, respectively, with addition of HDTMA⁺ 0.2M. These results indicate that there has been a change in the surface properties of the modified zeolite.

The next step is the adsorption test of the HDTMA⁺ modified zeolite against *Escherichia coli* and the *Staphylococcus aureus* bacteria, with a natural zeolite for comparison. According to [12], bacterial pathogens, viruses and protozoa tend to have a negatively charge under the pH conditions of groundwater. According to preliminary tests and adsorption results of the dichromate ions in the previous stage, the zeolite-HDTMA should also be able to adsorb *Escherichia coli* and *Staphylococcus aureus* bacteria. The bacterial adsorption performed at the dose, pH and contact time variations of the zeolite gave the optimal conditions, namely pH 7, a zeolite dose of 3 g and a contact time of 4 h. Adsorption with natural zeolite can adsorb only 5.6% of *S. aureus* and 7.2% of *Escherichia coli*, while modified zeolites can adsorb *S. aureus* bacteria up to 100% and *Staphylococcus aureus* up to 99%.

Table 1: The results of the adsorption activity of *Escherichia coli* and *Staphylococcus aureus*

| Sample | Adsorption of Bacteria (CFU/mL) | |
|--------|---------------------------------|------------------------------|
| | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> |
| ZMS | 6.8 x 10 ¹¹ | 1.8 x 10 ¹² |
| ZA | 5.0 x 10 ¹⁰ | 1.0 x 10 ¹¹ |

Natural zeolites have very little adsorption capacity compared to modified zeolites. The improved adsorption capacity of the modified zeolites is strongly influenced by the change in charge on the surface of the zeolite, from a negative charge to a positive charge, capable of binding to a pathogenic bacterium which tends to be in nature as a negative charge [2]. It is possible to reduce the concentration of bacteria in the adsorption process by using natural zeolites because the bacteria are trapped in the pores of the zeolite. The adsorption results of *Escherichia coli* and *Staphylococcus aureus* emphasize that there is an electrostatic interaction between the surface of the positively charged zeolite and the negatively charged bacteria.

IV. Conclusion

Analysis with SEM and FTIR shows that the modification was successfully carried out by modifying the zeolite surface structure and the appearance of peaks at 2850.68 cm⁻¹ and 2919.63 cm⁻¹. Natural zeolites and modified zeolites were able to adsorb consecutively 5.6 and 100% of *Staphylococcus aureus* bacteria and 7.2 and 99% of *Escherichia coli* bacteria at 3 g under zeolite assay conditions, and the concentration of *Staphylococcus aureus* bacteria at 1.8 x 10¹² CFU / mL and *Escherichia coli* bacteria at 6.9 x 10¹¹ CFU/mL.

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