

A Review antibacterial

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A Review: Antibacterial Activity of Metal Impregnated Zeolite in Water Treatment Process

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Abstract

Environmental and health problems related to water pollution by bacteria are a big challenge in the water treatment process. This pollution is caused by industrial development and increased population growth. Bacterial removal methods in water are continuously developed to find an efficient water treatment process. Zeolite has the potential to be an adsorbent used in the water treatment process. Zeolite has been widely used in adsorption and catalytic processes. Zeolite is a porous alumina silicate consisting of silicon, aluminium, and oxygen in its framework. Zeolite cations can be exchanged with monovalent or divalent ions. Zeolite does not have antibacterial activity, so it should combine with metal cations such as Ag, Cu, Zn, and Fe. Metal active that loaded into zeolite framework is a solution to improve the zeolite performance for bacterias removing in water. The impregnation method is the most widely used for the metal loading process into zeolite framework, because of its simple and economical process. This review will discuss natural and synthetic zeolites, types of metal, impregnation process, the antibacterial activity of metal impregnated zeolite, bacterial attack mechanism, and antibacterial application of metal impregnated zeolite in water.

Keywords: antibacterial activity, impregnation, metal, zeolite

1 Introduction

Water is a valuable resource necessary for life on earth. Water scarcity and water pollution are global problems. Industrial development and rapid population growth have resulted in high demand for water resources [1]. Monitoring groundwater quality is essential because most of the world's population uses groundwater for drinking [2,3]. The main problem of water pollution is its bacteria, which can affect health if consumed by humans [4]. Bacteria contaminating water usually come from domestic and industrial waste [5]. The primary indicator of water pollution is the presence of fecal pathogens such as *Escherichia coli* (*E. coli*) and *Enterococci*, which are often found in healthy water or river water, as a source of drinking water for the general public [6]. *E. coli* is the predominant bacteria in contaminated water. It is considered an indicator of drinking water's

hygienic condition, suitability, and safety [7]. More than 3.4 million people, primarily children, die each year from consuming water contaminated with bacteria. About 80% of diseases, especially in developing countries, result from bacteria-infected water [8]. Bacterial contamination of drinking water can cause many diseases and epidemics, for example, cholera, schistosomiasis, shigellosis, and typhoid fever [9].

Several methods have been carried out to overcome water pollution by bacteria, both chemically and physically [10]. The most commonly used processes are chlorination, UV radiation, and reverse osmosis using membranes. The chlorination method is efficient in causing the death of pathogens, but in the presence of organic matter, it produces trihalomethanes and haloacetic acid, which are highly carcinogenic compounds.



Component contamination can gradually interfere with the disinfection efficiency of the UV radiation process. Moreover, in the case of membranes, UV radiation is an expensive process [7]. The exchange process is an effective method [11,12]. This process is also favored because of its simplicity and economic advantages [13].

Currently, zeolites are widely used in the agricultural, medical, and chemical industries, such as adsorption, ion exchange, and catalysis processes [14–16]. Zeolite is considered a promising material for the water purification process [17]. Zeolites have a regular pore system and large surface area, high thermal properties, and chemical stability and are non-toxic [5,14,18,19]. Zeolite has the property of reversible ion exchangeability [7,17]. The crystal structure of zeolite has a negative charge and is interconnected so that it can absorb positively charged ions. Most toxic substances have a positive charge, so these toxic substances can be trapped in the zeolite framework [16].

Zeolite is considered a promising and essential material because it has a high ion exchange capacity [20]. Zeolite has no antibacterial activity [21]. The substitution of Si atoms by Al atoms in the zeolite framework creates a negative charge which must be compensated by the cations (the most common compensating cation is Na^+). These cations can be easily exchanged with metal cations Ag, Cu, or Zn to become zeolites with antibacterial, antiviral, and antifungal activity [18]. When cations with antibacterial properties are in the zeolite cavity through an ion exchange process, a material with antibacterial activity is obtained and can be an efficient antibacterial agent [19,21]. The study of the application of metal impregnated zeolite showed positive results for the water purification process [18].

This review discusses natural zeolite that can be formed naturally and synthetic zeolite that is intentionally made for commercial purposes, the metal impregnation process into zeolite framework, and several types of metal used in/metal impregnation process in antibacterial applications, the antibacterial activity of zeolite. Impregnated metal, further discussion of the mechanism of bacteria attack by metal will also be discussed in this review. At the end of the review will discuss the application of zeolite/metal as an antibacterial in water.

1.1 Natural Zeolite and Synthetic Zeolite

Natural zeolites are formed due to volcanic activity and are found in volcanic rocks. Natural zeolites have high selectivity to adsorb metals and

ammonium [22]. Zeolites in large quantities in nature are clinoptilolite, mordenite, chabazite [22], and phillipsite [23]. Clinoptilolite is the most abundant natural zeolite and is widely used to adsorb harmful compounds in water [23]. Clinoptilolite is not toxic to humans. It can even be used as an adjuvant in medical treatment [7,24].

Indonesian natural zeolites are scattered in 47 locations [25]. Geologically, zeolites are spread from the islands of Sumatra, Java, Kalimantan, Sulawesi [26], and Nusa Tenggara [27–29]. Indonesia has natural zeolite reserves of around 447,490,160 tons [29]. Two locations that have the largest zeolite resources are Sangkaropi (Tana Toraja) of 168,480,000 tons and Pasirgombang (Bayah) of 123,000,000 tons [30].

The most common zeolites in Indonesia are analysis, chabazite, clinoptilolite, heulandite, erionite, ferrierite, laumontite, mordenite, and flipside [30]. Natural zeolite from Sukabumi has three mineral compositions, namely quartz, mordenite, and clinoptilolite, but tends to be mordenite zeolite [26,30]. Malang natural zeolite and Gunung Kidul natural zeolite have a mordenite structure [31]. Zeolite from Wonosari Gunung Kidul is a type of mordenite and clinoptilolite with a SiO_2 content of 65.56% and Al_2O_3 of 11.04% [32].

The formation of natural zeolites takes a long time, while synthetic zeolites can be prepared in a short time [33]. The use of natural zeolite is also limited due to its low adsorption capacity [23]. For most applications, synthetic zeolite is preferred because it does not cause contamination which reduces the effectiveness of the zeolite [33] and shows better thermal stability [34]. In environmental engineering, synthetic zeolites are used in agriculture, medicine, and industry, such as removing heavy metals and other contaminants in water and gas purification processes [35]. The application of zeolite in petroleum processing shows that the absorption limit of synthetic zeolite is higher than that of clinoptilolite [22].

Synthetic zeolite is very sensitive to gel composition, temperature, and time in the manufacturing process. Controlling these parameters is very important to obtain high purity zeolites [33]. The suitability of the zeolite can be adjusted by decreasing or increasing the Al content or by maintaining the Si/Al ratio. Various synthetic zeolites are made depending on the Si/Al ratio. Because it has a more stable structure, synthetic zeolites are more expensive than natural zeolites [22]. The most common zeolite



frameworks are FAU, MFI, BEA, MOR, CHA, FER, and LTA [33].

Synthetic zeolites can be made from various unconventional sources of silica and alumina, such as kaolin, diatomite, coal fly ash, and rice husks. Zeolite A, X, and P can be synthesized from kaolin, zeolite A, X, Y from diatomite, zeolite NaX, NaY, NaP from coal fly ash, and zeolite NaX, ZSM-5, NaP from rice husk ash [23].

1.2 Metals for Impregnation Process

Silver (Ag) is a transition metal that exhibits high electrical and thermal conductivity. Ag is combined with various biomaterials in various chemicals to produce medical devices that aim to reduce or stop bacterial growth and infection by utilizing its antimicrobial properties. Ag-coated materials, alloy, and Ag buffer inhibit bacterial growth [36]. The zeolite that Ag has impregnated shows a smaller particle size and causes an increase in the surface roughness of the zeolite [3,37]. Ag has a very high price. Therefore, researchers are trying to find cheaper metal alternatives such as transition metals Cu and Zn which also exhibit antibacterial properties. The rate of bacterial removal from zeolites varied depending on the metal used in the following order $Ag^+ > Cu^{2+} > Fe^{3+} > Zn^{2+} > Ni^{2+}$ [18].

Cu's antimicrobial properties have been recognized for several years. They can be used to cure critical illnesses caused by fungi, germs in joints, intestinal tract, and urinary tract in the body system [38]. Currently, Cu is the most widely used material for domestic water distribution because it has outstanding corrosion resistance properties and reduces bacterial growth. Cu may also be used to control the development of *Legionella pneumophila*, the pathogen responsible for fatal pneumonia cases [39].

The results revealed that Cu containers could kill bacteria in drinking water [39]. Another research using the technique of incubation of drinking water in conventional media such as copper pots or glass bottles containing copper coils for one night showed that no bacteria (*E. coli*, *Salmonella typhi*, or *Vibrio cholera*) were present in drinking water [40]. The water purification technique with Cu-NP paper filters is a cheap way to purify water, so Cu-NP paper filters are suitable for improving water quality, especially in developing countries, where poor water quality can cause gastrointestinal diseases that affect many people in developing countries [41].

Cu impregnated zeolite showed better performance when compared to zeolite without Cu [42]. Cu is an essential metal for living organisms

at low concentrations but is toxic to most microorganisms and can prevent bacterial growth [21].

Zn is a vital metal for fungi. Although fungi need heavy metals such as Zn for their metabolic activity, Zn will be toxic in excessive concentrations [43]. Zn impregnation into zeolites A and X has anti-fungal characteristics [44]. ZnO can also be used in water purification processes. The process is simple, requires low cost, can be a potent antimicrobial, has a long service life, and is safe to use [45].

1.3 Mechanism of Bacteria Attack by Metals

Metals such as silver (Ag), copper (Cu), zinc (Zn), and magnesium (Mg) have been used as antibacterial long before the pharmaceutical antibiotic revolution [46].

Cell membrane degradation occurs through electrostatic interactions, disturbances in homeostasis through protein binding, reactive oxygen species (ROS) formation and oxidative stress, protein and enzyme disturbances, genotoxicity, and signal transduction inhibition. The inhibition of bacterial growth is achieved by either one or a combination of these mechanisms depending on the physical and chemical properties of the metals involved. Consequently, multi-action effects are possible by combining more than one metal type [47].

ROS can be lethal to bacteria because it destroys bio-organic molecules (amino acids, carbohydrates, lipids, nucleic acids, and proteins), lipid oxidation, protein modification, forms holes in the bacterial layer, inhibits enzymes, and causes RNA/DNA damage, which in turn eventually lead to cell death [1,47,48]. The mechanism of bacteria attack by metal can be seen in Figure 1.

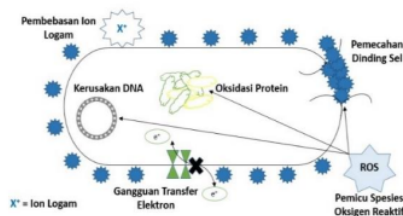


Figure 1. The mechanism of bacteria attack by metal.

Essential conditions for ROS formation are operating redox cycles, functional groups with oxygen groups on metal surfaces, and cell-particle interactions. Modification of properties and decrease in metal size also affect the regeneration of ROS. The interaction of the electron



donor/acceptor with oxygen allows the formation of superoxide anion (O_2^-), a Fenton-type reaction generating large amounts of ROS. The amount of ROS formed in bacterial cells is influenced by the physicochemical properties of the metal, such as surface area, diffusivity, surface properties, and electrophilic properties. ROS regeneration usually inhibits respiratory enzymes. Free radicals oxidize unsaturated phospholipids in cell membranes and cause more severe membrane damage. The integrity of the membrane is damaged due to lipid oxidation so that it interferes the function of the membrane. Cell membrane damage indicators, especially malondialdehyde, were increased due to lipid peroxidation by ROS [47].

In brief, the antibacterial mechanism begins with metal ions attached to the bacterial membrane through electrostatic interactions. It rapidly changes the condition of the bacterial membrane, which will result in bacterial death [49]. The antibacterial property of metallic silver is that as soon as silver ions are released and adhere to the bacterial wall, silver ions can bind to enzymes and proteins, causing severe damage to several functions in bacterial cells [50]. In addition, the formation of ROS can also occur due to the exposure of silver nanoparticles to oxygen. ROS can act as oxidizing agents of essential bacterial elements such as proteins, enzymes, and even DNA [51].

Antibacterial properties of Fe metal, where Fe metal is released and attached to the bacterial wall, will be adsorbed on the surface of bacterial DNA and oxidized through the Fenton catalytic process, where Fe will reduce intracellular hydrogen peroxide into reactive radicals. These reactive radicals will then attack and destroy the DNA chain, spreading further damage to the subcellular organs of the bacteria [46].

Other recent studies have also shown that the destructive ability of bacterial cell walls may stem from the interaction of metals with the peptidoglycan layer of the cell wall. The damaging effect of bacterial membranes can be strongly related to the physicochemical properties of the metal, such as the size, shape, and surface of the metal [46].

2 Method

Antibacterial preparation consisting of zeolite as a support material and metal as a precursor could be carried out using the impregnation method. The impregnation method is a chemical method for attaching an active precursor to a support material, then separating the

sample with a solvent, and final drying to convert the precursor into the desired shape [52]. This method depends on the interaction between the support surface and the metal in the precursor solution. This interaction resulted from the exchange of metal ions with hydrogen ions from the surface hydroxyl groups [53].

The impregnation process was carried out by mixing zeolite and solution, then stirring using a magnetic stirrer for 24 hours and filtering using deionized water. The samples were dried using a vacuum oven [54] and calcined using a furnace [55]. Several researchers executed additional treatments to maximize the performance of zeolites, such as contacting with *M. tuberculosis* leaf extract and NaOH [6], then contacting with HCl [21], or contacting with NaCl [7]. The antibacterial test was applied by preparing a bacterial culture with an age of 24 hours, making a bacterial suspension with Mac Farland I standard solution, counting the population of bacterial suspension as a negative control, preparing a sample solution then adding 1 mL of bacterial suspension, then homogenizing by vortex incubation at room temperature for 30 minutes, pouring 20 mL agar plate count medium and homogenizing, then letting stand for 2 x 24 hours at 35°C, counting the number of remaining bacteria [46].

There were two types of impregnation methods: dry impregnation and wet impregnation. Suppose the volume of the precursor solution was equal to or less than the volume of the pores of the support. In that case, this technique is known as dry impregnation, and this method relies more on retaining metal ions in the pores of the support during drying. The precursor solution is sprayed on the support in dry impregnation and seeps into the pores. The volume of the precursor solution does not exceed the pore volume of the support, and the time required for the impregnation process is shorter [53].

The volume of the precursor solution exceeds that required to fill the pores, a technique known as wet impregnation [53]. Wet impregnation involves the use of excess precursor solution. The time required for wet impregnation is much longer than for dry impregnation [52]. Wet impregnation was carried out systematically by adding more precursor solutions in the zeolite framework. The zeolite sample was added to the precursor solution. The mixture was then placed in a rotary evaporator to remove the water content. The samples were then dried and allowed to stand for one night under room conditions. Then the sample



was calcined in the furnace [43]. The metal loading efficiency by wet impregnation was about 70-75% [56].

The drying process carried out after the impregnation step was crucial in dry or wet impregnation. If the drying process was carried out too quickly, the liquid contained in the pores might be pushed out by the vapors formed deep

within the pores. This resulted in the deposition of the active phase on the outer surface of the support. This was undesirable because the metal may not adhere firmly enough to the support [53]. Table 1 describes the advantages and disadvantages of using dry and wet impregnation methods.

Table 1. Advantages and disadvantages of wet and dry impregnation methods.

Impregnation	Advantages	Disadvantages
Wet	<ol style="list-style-type: none"> 1. The method is simple, fast and generally used in inexpensive saline solutions. 2. Can store precursors on metal loading. 3. The amount of metal to be impregnated can be adjusted. 	<ol style="list-style-type: none"> 1. Use of excess volume of precursor solution. 2. The material is not deposited uniformly in the pores. 3. Deposited metal can be oxidized in a solution. 4. The time required is relatively longer.
Dry	<ol style="list-style-type: none"> 1. The volume of the precursor solution used can be equal to or less than the pore volume of the buffer. 2. The precursor and pore solution amounts are the same in the impregnation results. 3. It tends to retain metal ions when drying. 4. Shorter impregnation time. 	<ol style="list-style-type: none"> 1. Widely used in expensive salt solutions. 2. Rarely used and the process is a bit complicated. It must know the pore volume of the support first.

3 Result and Discussion

Absorption is one of the simplest and cheapest techniques for adequate water purification [8,57]. The adsorbent must be environmentally friendly, inexpensive, and efficient for various concentrations of different contamination [7]. One of the possible adsorbent solutions for water treatment is zeolites because zeolites have all the essential characteristics of environmentally friendly materials [4]. The ion-exchange properties of zeolites are used in various industries [58,59]. In recent years, they have been increasingly used to remove anions and cations from water due to their unique water treatment control, high ion removal efficiency, and processing speed [4]. Several researchers have studied the antimicrobial activity of using metal-impregnated zeolite in water purification. The results of this study can be seen in table 2.

Table 2. Antibacterial Activity of Metal Impregnated Zeolite in Water Purification Process

No	Material	Microbes	Antibacterial Activity	Ref
1	Cu/CLI	<i>E. coli</i>	Inhibition level: 100%	[60]
		<i>S. aureus</i>	Inhibition level: 59.26%	
		<i>C. albicans</i>	Inhibition level: 44.24%	
	Zn/CLI	<i>E. coli</i>	Inhibition level: 76.24%	
		<i>S. aureus</i>	Inhibition level: 5.56%	
		<i>C. albicans</i>	Inhibition level: 33.81%	
2	CA/AgNPs	<i>E. coli</i>	CFU = 0.1% (contact time: 2 hours)	[51]
	CA/Agβ		CFU = 0% (contact time: 2 hours)	
3	Cu/X	MRSA	MBC = 32 µg/mL (nano)	[61]



No	Material	Microbes	Antibacterial Activity	Ref
	Zn/X	MRSA	MBC = 64 µg/mL (micro) MBC = 512 µg/mL (nano) MBC = 512 µg/mL (micro)	
	Zn/X	MRSA	MBC = >10.000 µg/mL (nano) MBC = >10.000 µg/mL (micro)	
4	CuO/ZA	<i>B. subtilis B29</i> <i>S. choleraesuis</i>	Inhibition zone: 17 mm Inhibition zone: 14.55 mm	[20]
5	Ag/Z	<i>E. coli</i>	Total number of bacteria (CFU/mL H ₂ O) Biological treatment Contact time 25 minutes result: 12 Contact time 50 minutes result: 6 Contact time 75 minutes result: 2 Chemical treatment: Contact time 25 minutes result: 18 Contact time 50 minutes result: 12 Contact time 75 minutes result: 9	[6]
6	Cu/4A	<i>E. coli</i> <i>S. aureus</i>	MIC = 625 µg/L MIC = 1250 µg/L	[57]
7	Ag/FAU Zn/FAU	<i>E. coli</i> <i>E. coli</i>	Inhibition zone: 1,6 mm Inhibition zone: 0	[18]
8	Ag/Diatomite	<i>S. aureus</i> <i>K. pneumoniae</i>	MIC = 5 mg/mL (0.71% Ag) MIC = 2.5 mg/mL (4.65% Ag) MIC = 2.5 mg/mL (7.21% Ag) MIC = 10 mg/mL (0.71% Ag) MIC = 5 mg/mL (4.65% Ag) MIC = 5 mg/mL (7.21% Ag)	[62]
9	Ag/Y Cu/Y Zn/Y	<i>E. coli</i> <i>S. aureus</i> <i>E. coli</i> <i>S. aureus</i> <i>E. coli</i> <i>S. aureus</i>	MIC = 0.0657 mg/mL MIC = 0.135 mg/mL MIC = 0.122 mg/mL MIC = 0.276 mg/mL MIC = 1.161 mg/mL MIC = 0.566 mg/mL	[63]
10	ZnO/Chi-MCM	<i>E. coli</i>	Inhibition zone: 36 mm	[8]

Ag is a broad-spectrum antimicrobial agent. The antibacterial activity of Ag is recognized to be significantly superior. Antibacterial material made from Ag is suitable for water disinfection [7].

Ag-Zeolite (Ag/Z), which was biologically treated with the addition of *M. tuberculata* leaf extract (B-Ag/Z), revealed higher bactericidal activity than Ag/Z, which was chemically treated with the addition of NaOH (C-Ag/Z). This was because more silver doping on B-Ag/Z compared to C-Ag/Z. The antibacterial activity of B-Ag/Z,

which was higher than that of C-Ag/Z, was caused by silver doping on the surface of B-Ag/Z [6].

Ag impregnated FAU zeolite was applied as a membrane in the water purification process, showing a bacterial inhibition zone of 1.6 mm against *E. coli* bacteria, and no bacterial colony formation was observed. This showed bactericidal solid properties of Ag impregnated zeolite membranes, whereas pure FAU zeolite did not show antibacterial activity [18].



Bacterial solutions incubated with Zn²⁺ impregnated FAU zeolite membranes decreased bacterial colony formation activity, indicating good antimicrobial activity for these membranes. This revealed that Zn had the potential to be an antibacterial agent [18].

Besides Ag, Cu metal was also impregnated into the zeolite and analyzed for its antibacterial activity. Pure zeolite 4A had a MIC value of > 5,000 g/L, which indicates the absence of antibacterial activity. Cu impregnated zeolite 4A showed relatively high antibacterial activity against gram-negative and gram-positive bacteria. Cu/Zeolite 4A against *S. aureus* was two times higher than the antibacterial activity of Cu/Zeolite 4A against *E. coli*. The MIC values for *E. coli* were 625 g/L and 1250 g/L for *S. aureus* [57].

Reduction of bacteria using adsorbent made from natural zeolite clinoptilolite (CLI) activated Cu²⁺ (Cu/CLI) was quite effective against *C. albicans*, *S. aureus*, and *E. coli*, while for adsorbent activated Zn²⁺ (Zn/CLI), the most resistant bacteria was *S. aureus*, *C. albicans*, and *E. coli*. The highest level of inhibition was against *E. coli* bacteria, 76.24% (Zn/CLI) and 100% (Cu/CLI). The levels of inhibition against *S. aureus* bacteria were 5.56% (Zn/CLI) and 59.26% (Cu/CLI). The levels of inhibition against bacteria *C. albicans* were 33.81% (Zn/CLI) and 44.24% (Cu/CLI) [60].

Zeolite Y (ZY) impregnated with Ag, Cu, and Zn metals were also applied as nanofibre membranes and analyzed for their antibacterial activity against *E. coli* and *S. aureus*. It was found that Zn-Cu-Ag/ZY nanofibres showed the largest inhibition zone of 12 mm against *S. aureus*, while the largest zone of inhibition for *E. coli* was performed by Cu-Ag/ZY, which was 14.67 mm. The metal loading in Zn-Cu-Ag/ZY was 28.42%, and the silver exchange in Ag/ZY was 33.65%. The lower metal exchange in the nanofibres may show a reduced toxicity effect with higher antibacterial efficiency. The antibacterial activity of Zn/ZY was the lowest for the studied bacteria. There was a smaller zone of inhibition for Gram-positive bacteria than for Gram-negative bacteria. This may be related to the high peptidoglycan cell wall thickness in Gram-negative bacteria reported in many studies [63].

4 Conclusion

Water made the earth superior to other planets. However, there were some areas where the water source was insufficient for basic needs, such as drinking water, due to many bacterial

contamination and pollutants. This required further research on the water treatment process. Metal impregnated zeolites have been widely studied for water treatment processes. The need for effective water treatment, particularly sterilizing drinking water, eliminating bacterial contamination, and increasing drinking water production through water treatment approaches that can be modified flexibly. Zeolite did not have antibacterial activity, but it functioned as a support for ions that can be used as antibacterial. The amount of metal was limited by the ion exchange capacity of the zeolite. The quantity and the type, and pore size of the zeolite were essential factors that affected the metal ion release properties so that it could affect the bactericidal effect. Metal impregnated zeolites have proven antibacterial properties in water purification processes. The impregnation process had the advantage of being a simple and economical process. The application of metal impregnated zeolites as adsorbents and membranes has proven to be very efficient in antibacterial activity in water.

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