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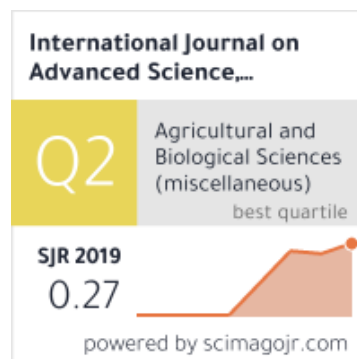
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Abstract—The TiO₂-Fe₃O₄-bentonite was used in this study as a photocatalyst material for antimicrobial. The material was coated on a ceramic container. This study is the preliminary study on coatings formulated using TiO₂-Fe₃O₄-bentonite to fight against microbial. This paper aims to emphasize the application of TiO₂-Fe₃O₄-bentonite in the water purification process by adding material into the paint and coating it on ceramic containers as a reactor to help neutralize *E. coli* and *S. Aureus*. The TiO₂-Fe₃O₄-bentonite powder was synthesized by the sol-gel method. The photocatalyst powder was exhaled on the surface of the inside painted-walls of the reactor. Some photo-catalytic parameters have been investigated, such as the photocatalyst concentrations and the initial concentration of *E. coli* starter, and *S. Aureus* starter. The result showed that the higher the concentration of the photocatalyst material, the more effective its degradation. Also, the highest death rate occurs when the initial concentration of the *E. coli* starter is at 107 CFU/ml. Photo-degradation in gram-negative bacteria (*E. Coli*) gives more promising results than the process in gram-positive bacteria (*S. aureus*). The characterization of the material showed that the photocatalyst material leached during the photo-degradation process. This causes the more extended the reaction takes place; there will be a decrease in bacterial photo-degradation activity. Also, the use of solar light in the photo-catalysis process is more effective than UV light.

Keywords— photo-catalytic; TiO₂-Fe₃O₄-bentonite; *E. Coli*; *S. Aureus*; antimicrobial.

I. INTRODUCTION

The distillation of water from chemical pollutants and micro-organisms is an essential process to extract pure water. Conventional water treatment uses chlorine as a disinfectant. In general, the stages of water treatment are natural filtration and sedimentation, coagulation and flocculation, sedimentation, filtering, and disinfection. However, residual chlorine contained in the water after the disinfection process is hazardous if consumed by humans because of the presence of a carcinogenic matter [1].

Nowadays, the photo-catalytic method is widely used in the degradation of dyes and bacteria. One material that is widely studied is TiO₂ due to its cost-effectiveness, chemical stability, high oxidizing ability, safety, and reusability [2]–[6]. Some researchers successfully showed the use of TiO₂ as photocatalyst [7]–[12]. *E. coli* is a harmful micro-organism which indicates the presence of bacteria in water. According to some studies, the use of photo-catalytic material with TiO₂ or other metal oxides can inactivate this toxic bacterium [13]–[27].

In our previous work, we reported the use of TiO₂-Fe₃O₄-bentonite as photocatalyst materials [28]–[31]. However, various problems tend to arise that need further treatment

during the degradation process. This study is, therefore, a continuation of our initial report [32] and also the preliminary study on coatings formulated using TiO₂-Fe₃O₄-bentonite to fight against microbial. This paper aims to emphasize on the application of TiO₂-Fe₃O₄-bentonite in the water purification process by adding material into the paint and coating it on ceramic containers as a reactor to help neutralize *E. coli* and *S. Aureus*.

II. MATERIAL AND METHOD

A. Materials

Ti-isopropoxide, *NH₄OH*, *FeCl₂.4H₂O*, *tetramethylammonium chloride*, *FeCl₃.6H₂O*, *ethanol* and *sodium hydroxide* were used as materials for synthesizing of *TiO₂-Fe₃O₄-Bentonite photocatalyst*. All these materials were purchased from Sigma-Aldrich. *Bentonite* clay was excavated from Pacitan, Indonesia.

B. Methods

TiO₂-Fe₃O₄-bentonite has been prepared according to the method described in our previous work [31], [32]. PANalytical PW 3373/00 X'Pert X-ray was used to observe the crystalline phase of the photocatalyst material (CuKα, 1.54 Å, radiation at 30 mA and 40 kV applied). At the same

time, SEM FEI INSPECT S-50 was used to observe the topography and morphology.

The photo-degradation of bacteria was carried out in a 12 x 7 x 3 cm ceramic container coated in water-based paint and sprayed with the photocatalyst material. The antimicrobial test was conducted by irradiating the bacterial suspension in a container with UV light at $\lambda = 325$ nm at a particular intensity for 180 minutes. 0.1 ml of the solution is taken every 30 minutes (t). The sample was diluted, and the total number of bacteria counted using a Total Plate Count (TPC) method. The parameters studied were preliminary bacterial concentration (103-107 CFU/mL, namely N_0), a type of gram bacteria, and UV light source. The results of this TPC method will be obtained from the unwanted bacteria after treatment (N_t). This would be acquired from the data at a constant rate of bacterial death, through the equation:

$$\ln \frac{N_t}{N_0} = -k_d \cdot t \quad (1)$$

The bacteria used are *E. coli* ATCC 25922, and *S. aureus* FNCC 0047.

III. RESULTS AND DISCUSSIONS

A. Photocatalyst Characterization

XRD characterization of photocatalysts has also been described in substantial details [31], [32]. The main peak on $2\theta = 30.3^\circ$ and 35.7° refer to the crystal of the magnetite phase, and these were supported by $2\theta = 30.3$ and 43.5° . That of $2\theta = 26.6^\circ$ referred to TiO_2 anatase phase. The intensity of anatase is smaller than the magnetite because *Ti* is less dominant than the *Fe*. While the peak at $2\theta = 9.9^\circ$; 13.0° ; 19.8° ; and 21.7° refer to bentonite. Anatase and magnetite are the most important phase in the photo-catalytic process [10].

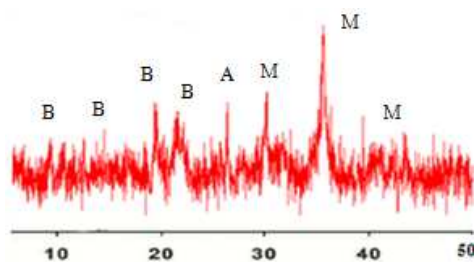


Fig. 1 XRD Photocatalyst Material Bentonite- TiO_2 - Fe_3O_4 , (A) Anatase TiO_2 , (B) Bentonite, (M) Magnetite Fe_3O_4

B. The *E. coli* and *S. aureus* bacterial growth

According to Llorens et al. [33], the growth curve of bacteria can be divided into 4 phases, namely lag phase, exponential phase, stationary phase, and death phase. These bacteria growth curve represented by the optical density (OD) obtained through a spectrophotometer. In Fig. 2a, the *E. coli* growth curve grew, up to the 27th hour, when only the exponential and stationary phases were obtained. The exponent phase of *E. coli* occurred from the first to the 12th hour. However, observations continue until the 27th hour when it reached the stationary phase of the growth of *E. coli*. In Fig. 2b, for the growth curve of bacteria *S. aureus*, it is apparent that there are four phases of growth discussed in the

literature, namely the lag, exponential, stationary, and death phases. This phase of growth occurs for 4 hours, followed by an exponential state, which lasts up until the 10th hour. Furthermore, the bacteria stationary phase ran until the 18th hour and lasted till the death phase observations were carried out at the 27th hour.

The growth curve of the bacteria was used to determine the hours it started entering the stationary or final phase, with those in the former used in carrying out this research. The reason for choosing this set of bacteria is because its growth rate equals the same as its mortality. The Photo-degradation of bacteria is not affected by an increase or reduction in the number of bacteria naturally.

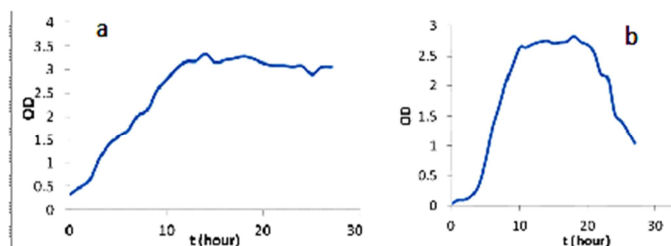


Fig. 2 (a) *E. coli* bacteria growth curve, (b) *S. aureus* bacteria growth curve

C. Photo-catalytic test of Bentonite- TiO_2 - Fe_3O_4 in terms of bacteria degrades

1) Effect of Concentration Photocatalyst Material Bentonite- TiO_2 - Fe_3O_4 : The experiments were performed using the initial conditions of *E. coli* bacteria in the early stationary phase, an initial concentration of about 10^6 CFU/ml, and photocatalyst material of 0.1 g/L and 0.2 g/L. Irradiation is conducted using UV light with a wavelength of 354 nm and intensity of 1.8 W/m^2 .

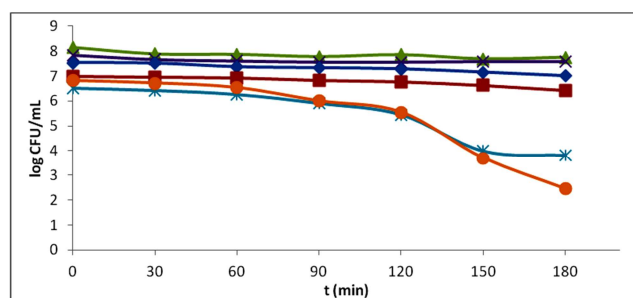


Fig. 3 Effect of Photocatalyst Concentration toward bacteria degradation (—■—) UV Control, (—●—) Bacteria Control, (—▲—) Material Control 0.1 gr/L, (—◆—) Material Control 0.2 gr/L, (—×—) Material Concentration Control 0.1 gr/L, (—*—) Material Concentration 0.2 gr/L

The UV control was conducted using UV light, and that of bacteria was done without using UV lamps and in a dark room. Material control is carried out in the absence of UV irradiation light. As can be seen from Fig. 3, an insignificant decrease in the number of bacteria in the UV control indicates that the UV lamps used do not have a significant effect on the degradation of the *E. coli*. On the control, the material indicates no significant reduction in the number of bacteria inherent. Therefore, the photocatalyst material does not influence on the bacterial degradation. Also, the photocatalyst concentration does not affect decreasing the number of bacteria death if not irradiated by UV light,

according to its constant rate values, as contained in Table 1 below.

TABLE I
DEATH RATE CONSTANT OF *E. COLI* BACTERIA (CONTROL)

| Control | Kd, mins ⁻¹ |
|--------------------------|------------------------|
| Material Control 0,1 g/L | 0,007 |
| Material Control 0,2 g/L | 0,006 |
| UV Control | 0,004 |
| Bacteria Control | 0,002 |

In Fig. 3, in both the photocatalyst concentration of 0.1 g/L and 0.2 g/L, the number of bacteria decreased significantly after 120 minutes. Between 0-120 minutes at concentrations of 0.1 g/L and 0.2 g/L, the difference is not too visible. The decline in the number of these bacteria can be concluded for their photo-catalytic effect of the material used, that when irradiated by UV light, will degrade the bacteria. Mortality rate constants of *E. coli* can be seen from Table 2 below. The use of photo-catalysts results in an increased mortality rate of the bacteria owing to the numerous numbers of photocatalyst material used, resulting in the formation of free radicals. Therefore, the value of the rate constants for bacterial death concentration of 0.2 g/L is higher than that of *E. coli* degradation test with a concentration of 0.2 g/L.

TABLE II
DEATH RATE CONSTANT OF *E. COLI* BACTERIA (PHOTO-CATALYST)

| Photocatalyst concentration (g/L) | Kd, mins ⁻¹ |
|-----------------------------------|------------------------|
| 0,1 | 0,058 |
| 0,2 | 0,118 |

2) *Effect of Initial Concentration of E. coli Bacteria*: Fig. 4 shows that the three controlled trials, a significant decline in the number of bacteria, thereby decreasing the number caused by the photo-catalytic reaction.

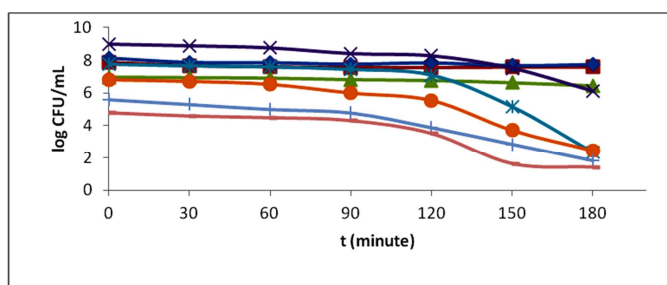


Fig. 4 Effect of Initial Concentration Bacteria *E. coli* toward Degradation Bacteria, (—♦—) Control UV, (—■—) Control Bacteria, (—×—) Control Material 0,2 gr/L, (—▲—) Concentration 10⁸ CFU/mL, (—□—) Concentration 10⁷ CFU/mL, (—△—) Concentration 10⁶ CFU/mL, (—○—) Concentration 10⁵ CFU/mL, (—●—) Concentration 10⁴ CFU/mL

The number of bacteria is decreasing as shown in Fig. 4; each initial concentration has the same trend, leading to a limited number of decreases in the number of bacteria in the first hour. After an hour, there is a significant amount of decrease in the number of bacteria. This result is because, in the bacterial cell, there are two kinds of enzyme, the catalase (CAT) and superoxide dismutase (SOD). These two can convert free radicals that attack bacteria into harmless components, thereby reducing the photo-catalytic effect.

However, the defense of bacteria by the two enzymes will be reduced and depleted due to the increasing number of free radicals formed from the photocatalyst material caused by UV light. This damage and destroys the bacteria after the first 1 hour. The death rate constants value of *E. coli* bacteria can be seen in Table 3 below.

TABLE III
DEATH RATE CONSTANT OF *E. COLI* BACTERIA (EFFECT OF BACTERIA CONCENTRATION)

| Bacteria Concentration (CFU/mL) | Kd, mins ⁻¹ |
|---------------------------------|------------------------|
| 10 ⁸ | 0,083 |
| 10 ⁷ | 0,181 |
| 10 ⁶ | 0,118 |
| 10 ⁵ | 0,076 |
| 10 ⁴ | 0,081 |

The death rate of bacteria is equivalent to its initial concentration (Fig. 4). The lower the concentration, the shorter the time required, and the higher the concentration, the longer the time needed. The higher the initial bacteria concentration, the longer the time required for photo-degradation activity. This result is due to the total bacteria in the river is about 10³-10⁵ CFU/mL, and compared to the stationary phase (± 10 hours), photo-catalytic material Bentonite-TiO₂-Fe₃O₄ is very effective to degrade in just about 3 hours.

3) *Effect of the Gram Bacteria on the Degradation Process*: In this study, two types of gram bacteria were used, i.e. *Staphylococcus aureus* as gram-positive and *E. Coli* as gram-negative bacteria.

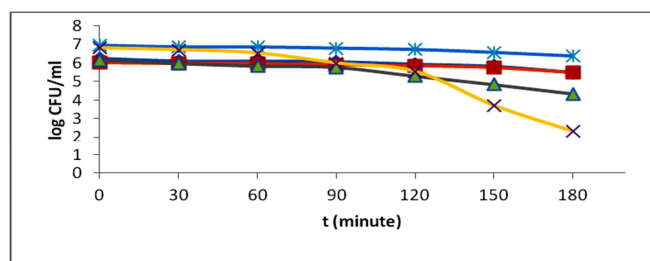


Fig. 5 Degradation activity test on different types of bacteria, (—♦—) Control bacteria Sauer, (—■—) Control UV, (—×—) Control Material, (—▲—) Bacteria *S. aureus*, (—□—) Bacteria *E. coli*

In Fig. 5, it can be observed that the control of bacterial, material, and UV, is relatively constant. Therefore, it can be concluded that in the control process, the bacteria are not degraded. In the bacteria *S. aureus*, the curve is constant for 90 minutes (Fig 5). Meanwhile, when compared with *E. coli*, the curve looks constant for 60 minutes. Furthermore, to have its defense system to attack from a harmful substance, the structure of the cell wall will serve as resistance against any harmful substance. Its cell wall is composed of three kinds of layers, the outer membrane, peptidoglycan, and inner cell membrane. Peptidoglycan on the gram-negative is about 2-7 nm, which is relatively thin. In gram-positive bacteria, the cell wall primarily consists of two sections, namely the peptidoglycan layer and the inner membrane. Its peptidoglycan layer is thicker than that of gram-negative, which is 20-80 nm. In this photo-catalytic process, ROS generated will make direct contact with the cell wall of the

bacteria; the gram-positive bacteria have a stronger resistance when compared to that of the negative. When ROS oxidizes with the bacterial cell wall, it is broken, and the cells will suffer from lysis, ROS will go further into the interior of the cell, which then oxidizes Coenzyme-A. This is very important to produce protein synthesis and respiration in the bacteria so that if the part is damaged, the cell will die.

TABLE IV
DEATH RATE CONSTANT VALUE OF *E. COLI* AND *S. AUREUS*

| Bacteria | Kd, mins ⁻¹ |
|------------------------------|------------------------|
| <i>Staphylococcus aureus</i> | 0,036 |
| <i>Escherichia coli</i> | 0,118 |

From Table 4, the value of the rate constant of *S. aureus* bacterial death is smaller than *E. coli*. Therefore, the degradation effect caused by the photocatalyst material Bentonite-Fe₃O-TiO₂ is more effective to *E. coli*.

4) *Effect of Solar Irradiance on the Degradation Process:*
In this research, the effectiveness of the photocatalyst material (Bentonite-Fe₃O₄-TiO₂), in the degrading bacteria was conducted using solar radiation. The research was performed using *E. coli* bacteria at the beginning of the stationary phase, with an initial concentration of 10⁶ CFU / mL. The experiment was conducted in August 2019 from 10:30 to 13:30.

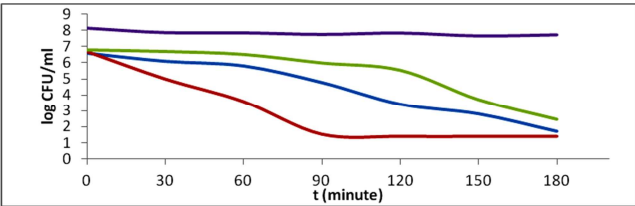


Fig. 6 Photo-catalytic degradation test of *E.coli* using solar radiation
(—) Control UV, (—) Photocatalysis using UV, (—) Control Solar radiation, (—) Photocatalysis using solar radiation

Fig. 6 shows the control from the solar radiation alone without any photocatalyst material, which results in the number of bacteria, is also decreased significantly from the concentration of 10⁶ CFU/ml to ± 50 CFU/ml in 180 minutes. Meanwhile, if the photocatalyst material is applied, with an initial bacterial concentration of 10⁶ CFU/ml, it decreases to 0 CFU / ml only within ± 90 minutes. The continuous line on the results with solar radiation above indicates that the bacteria have degraded. This result happens because when the water temperature is exposed to solar radiation, it increases up to 42°C so that *E. coli* can be degraded [20]. Additionally, the intensity of the sun reaches 80-90 W/m² on the date and time of the experiment. This result exceeded the reaction occurrence limit, thus causing degradation of bacteria significantly.

The intensity of sunlight is not in a constant condition because it depends on the weather, time, and season. The photo-catalytic process solar radiation allows bacteria to flourish again after the irradiation process. Additionally, some pathogenic bacteria are resistant to solar radiation. The sunlight has a wide wavelength range, from 310-2300 nm, capable of generating ROS is the range of 320-450 nm.

There is a constant bacterial death with an insignificant difference to the UV obtained from the sun comparing the value of kd (Table 5). Even though in theory, the value of kd by UV photo-catalytic process should be higher when compared with that of solar radiation. The result above is because of the massive intensity of solar light which causes death rate value equating to that of UV radiation.

TABLE V
DEATH RATE CONSTANT VALUE OF *E. COLI* (EFFECT OF RADIATION)

| Type of Light Irradiation | Kd, mins ⁻¹ |
|---------------------------|------------------------|
| UV | 0,118 |
| Solar | 0,128 |

D. The Chalking Effect of Wall Paint’s Binder Due to the photo-catalytic reaction
Photo-catalyst Material Bentonite-TiO₂-Fe₃O₄

Scanning Electron Microscopy (SEM) in this study was conducted to observe the surface structure of Bentonite-Fe₃O₄-TiO₂ before and after the photocatalyst.

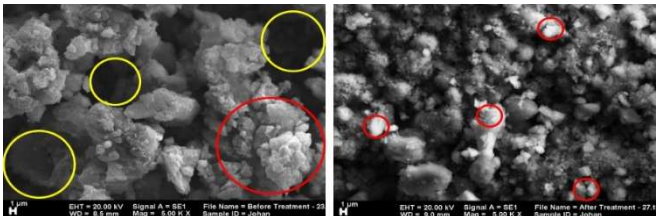


Fig. 7 SEM morphology of the surface of Materials Photocatalyst Bentonite-Fe₃O₄-TiO₂; (a) Before, and (b) After Photocatalytic Reaction; (○) Pores of Bentonite, (○) Materials TiO₂ and Fe₃O

In Fig. 7a, before the material was used to test the applications, paint binders could be seen covering the surface of the catalyst. However, after its utilization, the bentonite pores are mostly closed and more scattered. This is due to damage to the paint binder.

TABLE VI
EDX CHARACTERIZATION OF MATERIAL PHOTOCATALYTIC BENTONITE-TiO₂-Fe₃O₄ BEFORE AND AFTER PHOTOCATALYTIC REACTION

| Element | Weight% | |
|---------|-----------------|----------------|
| | Before reaction | After reaction |
| Ti | 1.94 | 0.60 |
| Fe | 8.17 | 1.23 |

According to the EDX characterization, after the photocatalyst material was used in degrading the bacteria, the Ti and Fe content were reduced, as seen in Table 6. This result happens because when the procedure progresses, the resultant ROS manufactured by the photocatalyst can degrade the binder and pigment as an organic substance. This process is known as chalking effects or calcification. Damage to the paint pigment and binder, which resulted in the production of Ti and Fe, and initially bonded with a binder, becomes loose and dispersed. The liberation of TiO₂ makes the material becomes more widespread, as shown in Fig. 7b. The pores on bentonite are covered by the binder and paint pigment with some wasted as a result of the water in the tub made the content of Ti and Fe reduced.

IV. CONCLUSION

Photocatalyst method using bentonite material-Fe₃O₄-TiO₂ with the aid of UV light can degrade *E. coli* and *S. aureus*. The optimum concentration of the photocatalyst material in the degrading bacteria was 0,2 gr/L. By using a material concentration 0,2 gr/L, the highest rate constants of *E. coli* bacteria were obtained at 10⁷ is equal to 0.181 L/min. The higher the initial concentration of bacteria, the longer the time it takes to degrade the bacteria. The photo-catalytic process is more effective in degrading gram-negative bacteria compare to a gram-positive. The use of solar light in the photo-catalysis process is more effective than UV light.

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