The Effect of TiO$_2$ additives on the antibacterial properties (Escherichia coli and Staphylococcus aureus) of glaze on ceramic tiles

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Abstract. Antibacterial glaze coating on ceramic tiles with TiO$_2$ additive was conducted to meet environmental sanitation needs. Raw materials used were commercial glaze 107, silica sand, and 0-5% weight of TiO$_2$ powder. The glaze mixture was added with distilled water and ground in an alumina pot mill for 24 hours. The glaze mass was coated on the green body, dried and then burned at a temperature of 1200°C with a holding time of 2 hours. On the glazed tiles, there was a layer of glass with an amorphous structure based on the X-Ray Diffraction results. The addition of TiO$_2$ caused an increase in opacity but decreased visual white color. Glaze antibacterial properties were tested for Escherichia coli and Staphylococcus aureus bacteria using the disc diffusion method. The addition of TiO$_2$ caused resistance to two types of bacteria, both for raw and burned glaze. The TiO$_2$ inhibition was similar to amoxicillin as a positive control. It had a greater resistance to S. aureus than E. coli bacteria. The largest inhibitory diameter value of 7.7 mm obtained by 5% TiO$_2$ glaze powder burnt against S. aureus bacteria. Glaze coating on the tile caused a greater inhibition diameter of 24.75 mm for 1% TiO$_2$ burned glazed.

1. Introduction

Recently, pollutants and bacteria that can harm humans have been growing both in type, quantity and quality. Bacteria evolved to be more resistant and able to develop faster [1]. The spread of bacteria can occur in our environment, so that many industries are competing to launch products that are claimed to have antibacterial properties, including ceramic tiles. Antibacterial ceramic tile is necessary especially for hospitals, schools, restaurants, industrial facilities, public places, and playrooms [2,3].

Antibacterial ceramic tiles can be obtained by applying an antibacterial glaze or coating. Glaze is an impervious layer or coating of a vitreous substance which has been fused to a ceramic body through firing. Antibacterial glaze is obtained by adding active substances such as TiO$_2$, ZnO, Ag, Cu, its composite, etc [2-11]. In this study, TiO$_2$ was used as an active ingredient to obtain an antibacterial glaze. Titanium oxide is usually used in the manufacture of glazes to provide color and as an opacifier.

Hazmaliza [4] has examined the antibacterial properties of glazed tiles with titanium dioxide as an antibacterial active ingredient. Antibacterial properties were tested against Escherichia coli and succeeded in reducing the growth of the E. coli colonies even to death for 10% TiO$_2$ nanoparticles after 2 hours. However, the resulting tile glaze showed cracks for the addition of 10% and 15% TiO$_2$. Therefore in this study, a maximum of 5% TiO$_2$ was added to minimize glaze cracking. In addition, antibacterial tests were carried out on two types of pathogenic bacteria, namely E. coli which...
represents gram-negative bacteria and *Staphylococcus aureus* which represents gram-positive bacteria. *E. coli* bacteria play a role in the process of putrefaction, but if found in excess amounts in humans it causes diarrhea and fever. Bacteria *S. aureus* causes serious skin conditions such as boils, pimples, and itching [12]. The disk diffusion method (Kirby-Baeur) was used to analyze the antibacterial properties of both types of bacteria. This method tests the sensitivity limit of the active substance (titanium dioxide) to bacteria based on the ability of the active substance to diffuse in solid media that has been inoculated by the bacteria [13].

2. Materials and methods

2.1. Glaze suspension production and its coating on tile

Materials used for the production of glaze suspension were technical grade materials, those were: 250g of glaze 107, 250g of silica powder, 400 mL of distilled water and active substance of TiO₂ which varies from 0% - 5%. All materials were mixed and ground for 24 hours using a pot mill which contained 1 kg of alumina ball. The glaze suspension was filtered with a 200 mesh filter and coated on raw tile by dipping method. The tiles that had been coated were dried in the wind and then put in an oven at 105°C for 4 hours. Besides being coated on the tile, the glaze suspension was dried in an oven at 110°C for 4 hours and then crushed with a mortar. Glazed tile and glaze powder were burned in an electric furnace with a combustion route 27-40°C: 1 hour, 40-220°C: 1 hour, 220-400°C: 1 hour, 400-580°C: 1 hour, 580-760°C: 1 hour, 760-940°C: 1 hour, 940-1175°C: 1 hour, 1175°C held for 1 hour, 1175-1200°C: 1 hour, 1200°C held for 2 hours, and 1200-40°C: 6 hours.

2.2. Test of antibacterial property of glazed powder and tile

The materials tested for antibacterial properties were glazed tile, dried glaze powder, and glaze powder which had been burned at 1200°C. They were suspended and then tested for antibacterial property aseptically. The disk diffusion method (Kirby-Baeur) was used to analyze the antibacterial properties of both types of bacteria namely *E. coli* ATCC 11229 and *S. aureus* ATCC 6538.

2.2.1. Device sterilization. All instruments used for antibacterial testing were washed at first, dried and sterilized in an autoclave at 121°C for 120 minutes. Sterile petri dishes were then wrapped in a paper.

2.2.2. The making of 2 (two) types of solid media. The first was Mueller Hinton Agar (MHA) for gram-positive bacteria (*S. aureus*). MHA media were made from a mixture of Mueller Hinton Broth (MHB) of 4.2 grams and Bacteriological Agar of 4 grams. The next was Nutrient Agar (NA) media for gram negative bacteria (*E. coli*). NA media were made from a mixture of Nutrient Broth of 1.6 grams and Bacteriological Agar of 4 grams. Each mixture of the two types of media was dissolved with 200 mL aqua bides in an Erlenmeyer and then homogenized and sterilized in a 121°C autoclave for 120 minutes. After that, the sterilized media was poured into a sterile petri dish. The petri dish were shaken in a counterclockwise way at first and then in a clockwise way 5-10 times. The media was left to solidify and stored in the refrigerator.

2.2.3. Liquid media. Mueller Hinton Broth (MHB) was made with a composition of 21 gram / 1L aqua bides for gram-positive bacteria. Nutrient Broth (NB) was made with a composition of 8 gram / 1L aqua bides for gram-negative bacteria. Each liquid media was made in Erlenmeyer and then homogenized and sterilized in an autoclave for 2 hours at 121°C.

2.2.4. Bacterial culture. Inoculated test bacteria were taken out 1 isolate using a sterile ose wire, then suspended into a test tube containing 5 mL of liquid media. Furthermore, the suspension of the test bacteria was incubated in an incubator for 24 hours at 37°C. Then, the turbidity of bacterial suspension was standardized as equal to 0.1 Mc Farland.
2.2.5. *Antibacterial test carried out using the disc diffusion method.* The suspension of test bacteria was taken out using a cotton swab and applied to the agar medium. Subsequently, the suspension of the positive and negative control test samples was dripped on different disk paper by 10 µL and left for some time. Then, the disc paper was placed regularly on the agar media, then incubated for 18-24 hours at 37°C. After that, it was observed and measured the inhibition zone formed using the calipers. Inhibition was determined by subtracting the diameter of formed inhibition zone by the diameter of disc paper (disc paper diameter = 6 mm).

2.3. *Characterization*

The instruments carried out to characterize the results of this study are: X-Ray Fluorescence (XRF) to analyze glaze raw material, X-Ray Diffraction (XRD) PANalytical X'Pert HighScore to analyze the types of formed crystal on the surface of glazed tile and glaze powder, Scanning Electron Microscope (SEM) Jeol to analyze the morphology of glazed tile surface.

3. *Results and discussion*

3.1. *Chemical analysis of glaze raw materials*

The glaze raw materials used were glaze 107, silica sand, and technical TiO$_2$. Glaze 107 is a transparent-type (colorless) commercial glaze. The main chemical compounds in glaze 107 are SiO$_2$ and Al$_2$O$_3$ with concentrations of 70.55% and 13.09%, respectively (table 1). The compound of SiO$_2$ is one of the acid oxides which can be used as a glass layer and hardener. Silica has a melting temperature of 1610-1710°C. The compound of Al$_2$O$_3$ (neutral oxide) was used as hardener and thickener for suspension glaze material to form a harder glaze layer. Besides that, there are basic oxide chemicals which can act as fusion agents in glazes [12]. The smelting material is K$_2$O 4.25%, CaO 3.04%, Na$_2$O 2.12%, and MgO 0.19%. Glaze 107 also has a yellowish brown color like clay because there is a chemical compound of Fe$_2$O$_3$ 0.772%.

The active material added to obtain antibacterial property was technical TiO$_2$ powder with TiO$_2$ content of 98.18% as shown in table 2. The TiO$_2$ powder had an anatase crystalline phase. Anatase is a TiO$_2$ crystal phase which shows the best characteristics as a photocatalyst, antibacterial and optoelectronic [14].

3.2. *Whiteness index analysis of glazed tiles*

After the glaze tile was burnt, the visual appearance of the surface was observed and the whiteness index was measured using a brightness meter. The brightness test worked by firing UV light/visible light on the surface of the glaze tile. The whiteness index was determined by lightness, i.e. the ability of the test material (tile surface) to reflect light. The whiteness index value ($W^*$) which is getting closer to 0 indicates the whiteness of the test material. The whiteness index value of glazed tile with variations of TiO$_2$ addition can be seen in table 3. The more TiO$_2$ powder added indicated the whiteness index value was getting away from zero, so the appearance was increasingly less white.

The brightness index test results were in line with the results of visual tests as shown in figure 1, the more TiO$_2$ was added, the color of glaze surface was more yellowish. This happened because of the change in the TiO$_2$ crystal phase from the anatase phase to the rutile phase at a temperature of 700°C. This rutile phase was very easy to form complex compounds with other elements in glaze materials such as SiO$_2$, Al$_2$O$_3$, and Na$_2$O.

3.3. *Mineral analysis of glaze powder and glazed tile*

Mineral analysis was carried out for dry glaze powder and glaze powder and glazed tile that were burned at 1200°C, with the addition of 1% TiO$_2$. Dry glaze powder (not burned) contained crystals that came from the used raw material. Dry glaze powder contained alpha quartz and anatase as shown in figure 2. Quartz alpha came from the silica sand used which was one of the SiO$_2$ crystals that were stable at room temperature. Anatase was a form of TiO$_2$ crystal derived from the used TiO$_2$ powder.
Table 1. The results of chemical analysis of glaze material.

| Components | Concentrations (% weight) |
|------------|---------------------------|
| SiO₂       | 70.55                     |
| Al₂O₃      | 13.09                     |
| CaO        | 3.40                      |
| MgO        | 0.109                     |
| Na₂O       | 2.12                      |
| K₂O        | 4.25                      |
| Fe₂O₃      | 0.772                     |
| TiO₂       | 0.0810                    |
| P₂O₅       | 0.0205                    |
| SO₃        | 0.0204                    |
| MnO        | 0.0109                    |

Table 2. The results of chemical analysis of TiO₂ powder.

| Components | Concentrations (% weight) |
|------------|---------------------------|
| TiO₂       | 98.18                     |
| Al₂O₃      | 0.0193                    |
| CaO        | 0.0143                    |
| MgO        | 0.0832                    |
| SiO₂       | 0.138                     |
| K₂O        | 0.238                     |
| Fe₂O₃      | 0.0205                    |
| Na₂O       | 0.0575                    |
| SO₃        | 0.0282                    |
| P₂O₅       | 0.261                     |

Table 3. Brightness (W*) test results of combustion of glaze tile in temperature of 1200°C with variations of TiO₂ addition.

| TiO₂ (%) | W* value |
|----------|----------|
| 0        | -5.92    |
| 1        | -7.36    |
| 2        | -11.10   |
| 3        | -29.80   |
| 4        | -47.22   |
| 5        | -54.59   |
Figure 1. Glaze tiles results from combustion with variations of TiO$_2$ addition from 0-5%.

Figure 2. XRD diffractogram of dry glaze powder.

Figure 3. XRD diffractogram of glaze tiles burned at 1200°C.
After being burned at 1200°C, glaze powder contained new crystals, those were cristobalite and rutile, in addition to the crystals found in the raw powder which were alpha quartz and anatase, as shown in figure 3. Cristobalite was formed due to changes in the quartz phase to tridymite (amorphous) and tridymite turned into cristobalite. As a result of heating at 800°C, quartz turned into tridymite and when the temperature was raised to 950°C, cristobalite is formed. Rutile was formed due to anatase heating at a temperature of 600-700°C until it transformed into rutile.

The XRD diffractogram pattern for glazed tiles with 1% TiO$_2$ burned at 1200°C can be seen in figure 4. There were diffraction peaks for anatase, rutile, mullite, and quartz-quartz. Mineral Mullite was formed from the combustion of feldspar and clay in raw tile material. Tile raw material was usually in the form of silica, feldspar, and clay. Mullite was formed from clay (Al$_2$O$_3$.2SiO$_2$.2H$_2$O) which underwent heating to a temperature of 1000°C, was going to release 2 molecules of water to obtain a mullite crystal (Al$_2$O$_3$.2SiO$_2$). The XRD diffractogram pattern of glazed tiles (figure 4) was different from the glaze powder (figures 2 and 3), where the peaks that occurred tended to be short with a widening pattern or crystal that occurred were generally amorphous. This was due to the glaze layer applied to the tiles which tended to be thin and fused, resulting in an amorphous crystal structure.

![Figure 4. XRD diffractogram of glaze tiles burned at 1,200°C.](image)

3.4. Morphological analysis
SEM analysis aimed to determine the morphology of glaze tiles surface of TiO$_2$ 1%. Morphological observation of glaze tiles using SEM with 5,000x magnification can be seen in figure 5a. It shows Titanium dioxide particles that were shaped like chunks and were disorganized.

Agglomeration or buildup of titanium dioxide particles occurred on the surface of the glaze tile. The factors causing irregularities in particle shape and particle buildup were inhomogeneous glaze suspension and uneven application of glaze. As a result, when the application of antibacterial testing on 4-sided glaze tiles had a different diameter of inhibition. In figure 5, it can be seen on the 0% TiO$_2$ glaze tiles that there were also titanium dioxide particles. This material was obtained from glaze 107 that had titanium dioxide as much as 0.0810%.

3.5. Test for antibacterial properties
In this test, samples of raw glaze powder, combustion glaze powder and glazed tiles were used. While the tested bacteria were *E. coli* gram-negative bacteria and gram-positive *S. aureus* bacteria. The
second reason, these bacteria were used because they can easily grow in general conditions. Aseptic test for antibacterial property was carried out to avoid contamination from other bacteria.

**Figure 5.** SEM result (a) glazed tiles of TiO$_2$ 1% and (b) glazed tiles of TiO$_2$ 0%.

**Figure 6.** (a) glaze 1% TiO$_2$ with *E. coli* bacteria; (b) glaze 1% TiO$_2$ with *S. aureus* bacteria; (c) 5% TiO$_2$ glaze with *E. coli* bacteria; (d) 5% TiO$_2$ glaze with *S. aureus* bacteria; (e) 1% TiO$_2$ glazed tile with *E. coli* bacteria; (f) 1% TiO$_2$ glazed tile with *S. aureus* bacteria.

In this antibacterial test, it needed a media to breed the desired bacteria. For *E. coli* bacteria, the culture was made in NB (Nutrient Broth) media because NB media is a medium that is commonly used for the growth of heterotrophic microorganisms. For *S. aureus* bacteria, the culture was made in the MHB (Mueller Hinton Broth) medium because MHB is resistant to gram-negative and selective to
gram-positive bacteria, then MHB is used for the growth of *S. aureus*. MHB contains casein hydrolyzate as a source of proline amino acids that can optimize the growth of *S. aureus* bacteria.

In testing the antibacterial property, two controls were used. Those were positive control in the form of amoxicillin and negative control in the form of sterile aquabides. Positive control served as a comparison of the antibacterial performance of glaze and glazed tiles with commonly used antibiotics. While negative controls were used to determine the effect of solvents on the growth of test bacteria because sterile aquabides were used in making glaze suspensions as solvents. Here is a picture of the results of the antibacterial test, see figure 6.

![Figure 6. Antibacterial test results.](image)

**Figure 6.** Antibacterial test results.

The results of the antibacterial test were tested qualitatively and quantitatively. The qualitative test was carried out by observing the clear zone which indicated the inhibition of bacterial growth due to the presence of the active substance TiO$_2$. The quantitative test was measuring the diameter of clear zone or the inhibitory bacteria. Glaze samples and glazed tiles were shown to have antibacterial properties that were characterized by the presence of clear zones until inhibition diameter values were obtained (figure 6). Antibacterial activity of powder and glazed tiles was due to the photocatalytic properties of TiO$_2$. TiO$_2$ had a band gap of 3.2 eV and could produce OH*, O$_2$*, and H$_2$O$_2$ from the ROS (Reactive Oxygen Species) reaction [4,16]. These radicals will react with organic compounds contained in the cell membrane (peptidoglycan wall) until the bacterial cell undergoes lysis. And Ti$^{2+}$ ions surround the peptidoglycan wall area and bind with organic compounds and lyse the bacterial cell wall. TiO$_2$ and ZnO compounds have the same antibacterial activity mechanism, namely through the ROS reaction which produces radical compounds (OH*, O$_2$*, and H$_2$O$_2$) that can damage bacterial cell walls. This mechanism can be illustrated in figure 7 [15,16].

The diameter of inhibition for *E. coli* and *S. Aureus* bacteria can be seen in table 4. The addition of TiO$_2$ caused an increase in the inhibitory power for both types of bacteria, both for raw and burnt glazes. The inhibition of the TiO$_2$ glaze burned decreased for a concentration of 1%. This was due to the phase transformation from part of the anatase TiO$_2$ to rutile due to the combustion process above 700°C, as confirmed by the XRD diffractogram in figure 4. However, for the 5% TiO$_2$ concentration, the decrease in inhibition did not occur because the remaining anatase phase was still high so that the decrease in activity due to rutile was not significant. Glaze coating that contained TiO$_2$ on the surface of ceramic tiles caused an increase in the inhibition of TiO$_2$ against both types of bacteria because with coating, the contact surface area between TiO$_2$ and bacteria became larger.

The inhibition of TiO$_2$ compounds against *S. aureus* bacteria was higher than *E. coli* for all glaze samples as shown in table 4. This was because the cell wall structure of gram-positive bacteria was simpler than -negative one, as shown in figure 7, so that it was easily lysed by radical compounds resulting from the ROS reaction. Gram-positive bacteria only had a thick peptidoglycan layer on their cell walls, whereas negative bacteria had an outer membrane layer consisting lipopolysaccharide (figure 8). Lipopolysaccharide molecules carried a negative charge so that the cell wall of gram-negative bacteria tended to be more negative than -positive one. The ROS reaction of TiO$_2$ produced several negatively charged species such as *OH* and O$_2*$. These radical compounds would be more difficult to
penetrate on the cell membrane of gram negative bacteria, so that these bacteria became more resistant [17].

**Table 4.** The diameter of inhibition of *S. aureus* and *E. coli* bacteria in glaze samples.

| No | Glaze Sample                                      | Diameter of bacterial inhibition |
|----|--------------------------------------------------|----------------------------------|
|    |                                                  | *E. coli* | *S. aureus* |
| 1  | Glaze powder with 1% TiO$_2$                     | 6.1       | 7           |
| 2  | Burnt Glaze powder at 1200°C with 1% TiO$_2$     | 6         | 6.5         |
| 3  | Glaze powder with 5% TiO$_2$                     | 6.2       | 7.1         |
| 4  | Burnt Glaze powder at 1200°C with 5% TiO$_2$     | 6.3       | 7.7         |
| 5  | Burnt glazed tile with 1% TiO$_2$                | 24.1 x 24.15 | 25.4 x 24.1 |

Note: Diameter paper disk = 6 mm  
Size of tile = 24 mm x 24 mm

**Figure 8.** Comparison of bacterial gram-positive and -negative cell wall structure [17].

4. **Conclusion**  
An optimum whiteness value of 5.92 was obtained for tiles without TiO$_2$. Addition of TiO$_2$ to the glaze showed antibacterial properties both for *E. coli* and *S. aureus*. Antibacterial properties of TiO$_2$ glaze against *S. aureus* bacteria were more active than *E. coli*.

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