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EFFECT OF ADDITION AS ANTIBACTERIAL AGENT IN *PORTLAND* CEMENT WITH DISC DIFFUSION METHOD

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ABSTRACT

One of the most widely use types of cement is *Portland* cement, but in a humid environment conditions it can cause bacteria to appear on the cement surface which causes health problem, therefore, it is necessary to modify cement with antibacterial properties. This study was conducted to determine the antibacterial effectiveness of TiO_2 in *Portland* cement with *Eschericia coli* bacteria as the evaluating bacteria The disc diffusion method was use to evaluate the antibacterial properties by varying the TiO_2 in *Portland* Cement by 0%, 1%, 3% and 5%, and carried out with UV irradiation. The results showed that cement-TiO_2 can inhibit the growth of *Escericia coli* bacteria with the best antibacterial effectiveness obtained in the addition of TiO_2 to *Portland* cement as much as 5% with UV irradiation for 60 minutes to produce an inhibition zona of 7.35 mm.

Keywords: TiO_2, *Portland* cement, Antibacterial, Disc Diffusion

1. INTRODUCTION

Cement is the most widely used material in the construction of modern infrastructure and buildings [1-3], besides that cement is widely used as a basic ingredient in mortar, plaster, and concrete. Cement is a starchy substance made with lime and clay and calcined as the main ingredient [4-6]. One of the most widely used types of cement is *Portland* cement, where this cement contains 95% cement clinker [7]. However, in environmental conditions that have high humidity levels can cause the surface of the cement structure to become moist. This condition can trigger the growth of microbes such as bacteria. One of the bacteria found on the wet cement surface is *Eschericia coli* [8-

10]. Which can cause health problems.Therefore the development of functional cement-based materials is a revolutionary way to create new architectures with antibacterial properties [11].

The use of nanoparticles as an innovative, inexpensive, simple, and efficient material has received a lot of attention because of its ability to minimize the number of microorganisms and the antibacterial and cleaning abilities of nanoparticles that can be used in mortar and cement compositions. The current research focus is on the use of Titanium dioxide (TiO_2) nanoparticles in cement compositions to induce unique features as antibacterial agents against *Escherichia coli* (E. coli) bacteria. It is known that TiO_2 has antibacterial properties against bacteria, *Candida albicans Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other bacteria. TiO_2 nanoparticles have advantages such as having good optical properties, stability, high refractive index, low cost and non-toxic [12-14]. Where TiO_2 nanoparticles can produce Reactive Oxygen Species (ROS) in the form of hydroxyl radicals (•OH-), and superoxide anions (•O^2-) which can damage bacterial cell walls [15,16].

There are several methods used in the process of making nanoparticles such as the sol-gel method, coprecipitation, hydrolysis One of the antibacterial activities of TiO_2 nanoparticles can be seen by the disc diffusion method [20], by looking at the inhibition zone for bacterial growth or by looking at the clear zone formed around the paper disc [21-23], then measured using a caliper [24].In a study conducted Maryani at 2020 determined the effect of TiO_2 on the antibacterial properties of ceramic glazes, and it was proven that the addition of TiO_2 to the glaze showed antibacterial properties against bacteria such as Eschericia coli and Staphylococcus aureus, with the addition of 5% TiO_2 was able to inhibit the growth of *Eschericia coli* bacteria was 6.3 mm and *Staphylococcus aureus* bacteria was 7.7 mm [20].

TiO_2 is well known for its potential applications in photocatalysis and photoelectrochemistry due to its excellent optical transmission. One of the applications of TiO_2 nanoparticles as an antibacterial, especially the anatase phase, has resulted in many nanostructured TiO_2 materials being used as construction materials[25,26]. This study was conducted to determine the antibacterial effectiveness of TiO_2 in *Portland* cement with *Eschericia coli* bacteria as the evaluating bacteria The disc diffusion method was use to evaluate the antibacterial properties by varying the TiO_2 in *Portland* Cement by 0%, 1%, 3% and 5%, and carried out with UV irradiation.

2. EXPERIMENTAL SECTION

2.1 Tools and materials

Tools needed beaker, erlenmeyer, dropper, stirring rod, spatula, measuring pipette, funnel, analytical balance, oven, furnace, petri dish, filter paper, disc paper, magnetic stirrer, autoclave, ultrasonic digital, ose needle , Vernier calipers. And the materials used are $TiCl_4$, Ethanol, NH_4OH, Aquades, *Portland* Cement, nutrient agar and *Eschericia coli* bacteria.

2.2 Procedures

2.2.1. Synthesis and characterization of TiO_2

A total of 7 ml of $TiCl_4$ was added to 100 ml of distilled water, and 70 ml of ethanol was added, then stirred using a magnetic stirrer for 30 minutes, then NH_4OH was added until a white precipitate was formed, then the precipitate was allowed to stand for 12 hours then the precipitate was filtered using filter paper and washed with distilled water. after that it was dried in an oven at 110ºC, and calcined at 400ºC for 4 hours[17]. The synthesized TiO_2 powder was characterized using XRD to determine its structure, size, and crystallinity.

2.2.2 Mixing TiO_2 in *Portland* cement

TiO_2 powder with variations of 1%, 3% and 5%, respectively, was mixed with 1 ml of distilled water and 2 grams of *Portland* cement, and then sonicated using digital ultrasonication to obtain a homogeneous TiO_2-cement paste, and characterization of Cement-TiO_2 the sonicated TiO_2 cement paste was characterized using SEM.

2.2.3. Antibacterial test against *Eschericia coli*

Previously, the agar media was made by weighing 2.8 g of nutrient agar dissolved in 100 ml of distilled water and stirred and heated until the material was completely dissolved. Then the solution is poured into an Erlenmeyer and tightly closed. After that, the media was sterilized using an autoclave at a temperature of 121ºC and a pressure of 15 psi for 15 minutes. After sterilization, the nutrient agar medium was poured into a petri dish and allowed to stand at room temperature until solidified, then streaked *Eschericia coli b*acteria on the agar medium.

Paper discs measuring 5 mm were placed or immersed in samples of cement paste-TiO_2 1 %, 3%, 5%, positive control, and negative control. Then the paper discs that have been saturated with the sample and control are placed into the media that has been spread with *Escherichia coli* bacteria on the agar media. Furthermore, the petri dishes were exposed to UV light for 0, 30 and 60 minutes to examine the effect of UV light on the antimicrobial potential of TiO_2 nanoparticles and then incubated. Then visually observed the results of antibacterial activity formed from the sample in the form of a zone of inhibition of bacterial growth around the paper disc and measured with a caliper. The following Table 1 shows the category of inhibition of bacterial growth [27-29].

Table 1. Categories of bacteria growth inhibiting power

Inhibitory Power of Bacteria(mm)	Category
< 5	Weak
5-10	Keep
10-20	Strong

>20	Very strong

3. RESULTS AND DISCUSSION

3.1 Synthesis and Characterization TiO_2

In the synthesis of TiO_2 nanoparticles, $TiCl_4$ solution was used as a precursor. Starting with mixing a solution of $TiCl_4$ with distilled water and then adding ethanol then stirred for 30 minutes while adding NH_4OH to get a thick white solution, adding NH_4OH functions as a precipitator to produce a thick white solution, after that it is allowed to stand for 12 hours and a solid precipitate is obtained is titanium hydroxide.The resulting precipitate was dried using an oven at a temperature of 110˚C to remove the moisture content then a calcination process was carried out for 4 hours at a temperature of 400˚C. After going through the calcination process, pure TiO_2 powder was obtained with a white and smooth form.

TiO_2 nanoparticles that have been synthesized, then characterized by XRD instruments are useful to find out information about the size, structure and crystallinity of the synthesized TiO_2 nanoparticles. In this study, the XRD pattern was obtained as shown in the following figure:

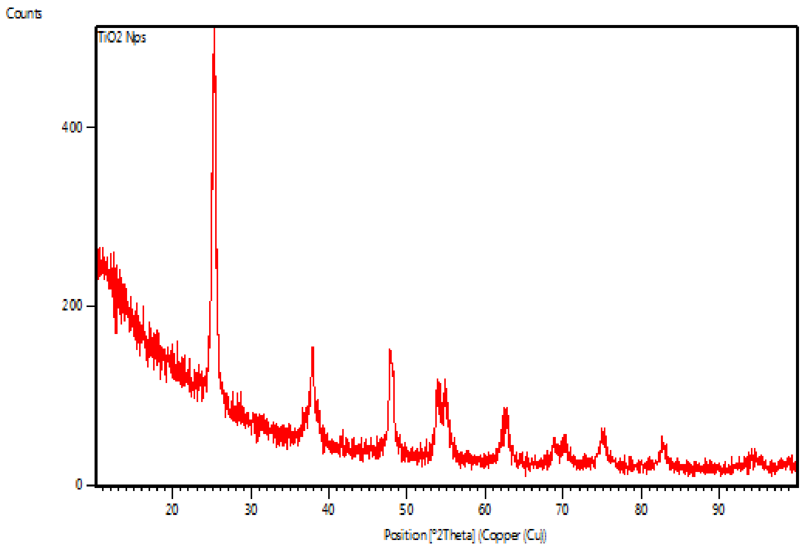

Figure 2. XRD Pattern of TiO_2

The *X-ray diffraction* pattern of TiO_2 shown in Figure 16 peaks were found at angles of 25.2114º, 37.8414º, 47.8566º, 53.8231º, 54.8247º, 62.7959º, 68.7692º, and 70.1783º. Among the XRD peaks at an angle of 25.2114º are peaks that have high intensity and based on the results of calculations using the Debye-Scherrer formula, the crystal size of TiO_2 is 15.732 nm.Intense sharp peaks indicate that the anatase crystal phase was successfully formed. The XRD pattern in the image proves the purity level of the synthesized TiO_2 crystal powder, strong diffraction peaks at 25.2114º and 47.8566º indicate TiO_2 in the anatase phase, and good degree of crystallinity. This indicates that the synthesized TiO_2 can be used for applications as a good antibacterial.

3.2 Characterization Cement-TiO_2

The surface morphology of Cement-TiO_2 was seen using the SEM instrument, which was carried out with a magnification of 10000x where it was seen that TiO_2 had a spherical almost spherical shape of TiO_2 morphology [17] And it looks like agglomeration or accumulation of TiO_2 particles occurs on the cement surface. SEM image results of cement-tio2 to see the surface morphology can be seen in Figure 3.

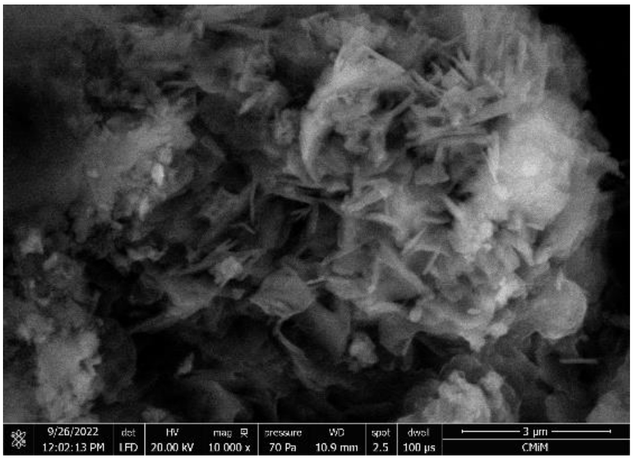

Figure 3. The SEM images of Cement-TiO_2

Factors that cause irregularity in particle shape and particle accumulation are less homogeneous cement suspensions and uneven cement application. forms such as calcium silicate hydrate gel [30-32], and calcium hydroxide in the form of sharp crystals which are compounds produced when mixing water with Portland cement or cement hydration products [33-35]. SEM results that there is no reaction that occurs between Cement and TiO_2 in the sample of Cement-TiO_2 and there is no formation of other phases in Cement-TiO_2.

3.2 Antibacterial Test Against *Eschericia coli*

3.2.1 UV irradiation Antibacterial Test no UV light

In antibacterial testing without the help of no UV light, there was no inhibition zone for bacterial growth formed in each variation of TiO_2 added to *Portland* cement as an antibacterial agent that did not work well. The absence of an inhibition zone for bacterial growth indicates that TiO_2 added to *Portland* cement as an antibacterial agent does not work properly in the absence of UV light bacteria.

3.2.2 UV Radiation Antibacterial Test 30 minutes

At 30 minutes of UV irradiation, a zone of inhibition of bacterial growth formed around the paper disc was seen, this indicates that Cement-TiO_2 has antibacterial properties when exposed to UV light. The diameter of the inhibition formed was measured by measuring the diameter of the resulting clear zone, then subtracted from the diameter of the 5 mm paper disc. So that the average diameter of bacterial inhibition is obtained as shown in Table 2.

Table 2. Results of measuring the diameter of the 30 minutes of uv resistance zone

No	Treatment(%)	Average Inhibitory Zone Diameter (mm)	Growth Barrier Response
1.	0	-	*Not response*
2.	1	3.13	Weak
3.	3	3.98	Weak
4.	5	4.23	Weak
5.	(+)	7.05	Keep
6.	(-)	-	*Not response*

From the results of the measurement of the diameter of the inhibition zone of bacterial growth formed at the addition of 1% and 3% and 5% TiO_2, it was still classified as weak based on the category of inhibition of bacterial growth according to [14], but in the positive control the inhibition of bacterial growth was in the medium category. The small inhibition zone formed does not mean that the antibacterial properties of TiO_2 are less active, but that the hydroxyl radicals and superoxide ions produced are slightly affected by the length of UV irradiation[40-42].

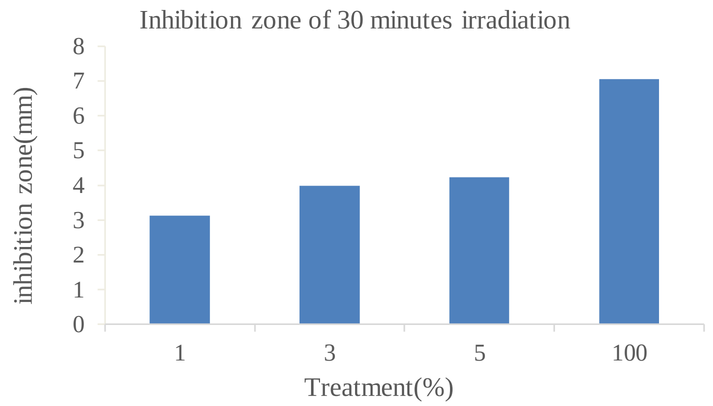

Figure 4. Response Resistance to 30 minutes irradiation

3.2.3 UV Radiation Antibacterial Test 60 minutes

In 60 minutes of UV irradiation, better results were obtained because TiO_2 added to *Portland* cement as an antibacterial was proven by the formation of an inhibition zone for bacterial growth around the paper disc, the inhibition zone formed was then measured using a caliper and the results can be seen in table 3.

Table 3. Results of measuring the diameter of the 60 minutes of uv resistance zone

No	Treatment(%)	Average Inhibitory Zone Diameter (mm)	Growth Barrier Response
1.	0	-	*Not response*
2.	1	6.18	Keep
3.	3	6.88	Keep
4.	5	7.35	Keep
5.	(+)	12.20	Strong
6.	(-)	-	*Not response*

The table above shows the size of the inhibition zone for the growth of Eschericia coli bacteria with variations in TiO_2 with the help of UV light for 60 minutes. The greater the variation of TiO_2 added, the greater the inhibition zone for bacterial growth formed, this indicates the greater the ability of TiO_2 to inhibit the growth of *Eschericia coli* bacteria. Where the inhibitory response to bacterial growth at concentrations of 1%, 3% and 5% was moderate, and the positive control had a strong inhibitory response to the growth of Eschericia coli bacteria. This is because TiO_2 has the ability to kill bacteria due to its photocatalytic properties by producing ROS where superoxide ions and hydroxyl radicals can destroy cell walls and cause bacterial death [20]. After UV irradiation on TiO_2, ROS (*Species Oxygen Reactive*) are produced, namely hydroxyl radicals (OH•) and superoxide anions (•O^2-) which first damage the cell wall layer, thus allowing the leakage of small molecules such as ions, and then ROS can penetrate cells more deeply. Away, causing degradation of internal components and leakage of molecules resulting in cell death [36-39].

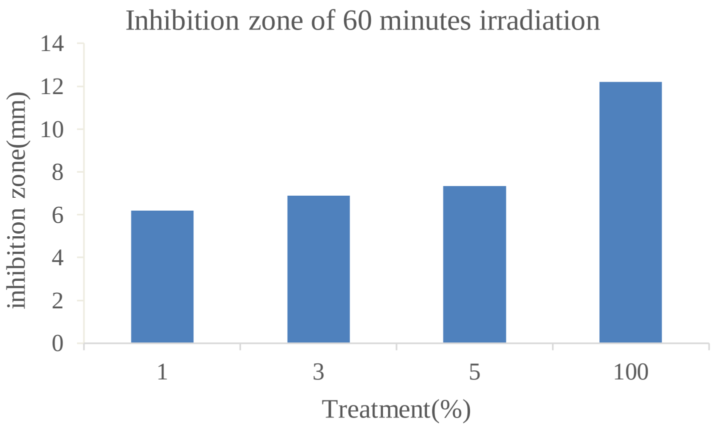

Figure 5. Response Resistance to 60 minutes irradiation

From the results obtained showed that with UV irradiation for 60 minutes increased the performance of TiO_2 as an antibacterial agent[43-44]. The longer the irradiation time, the more hydroxyl radicals (•OH-) and superoxide anions (•O_2-) produced and the greater the ability of TiO_2 nanoparticles to kill bacteria. Where TiO_2 nanoparticles can produce Reactive Oxygen Species (ROS) in the form of hydroxyl radicals (•OH-), and superoxide anions (•O^2-) which can damage bacterial cell walls [15,16].

Thus, the percent response of the growth inhibition of *Eschericia coli* bacteria to TiO_2 cement increased along with the increase in the number of TiO_2 variations added to 60 minutes of UV irradiation, and without the addition of TiO_2 can not inhibit the growth of bacteria.

4. CONCLUSION

Based on the research that has been done, it can be concluded that the modification of *Portland* cement into antibacterial cement with the addition of TiO_2 has been successfully carried out, the effect of adding TiO_2 can increase antibacterial effectiveness with the *Escherichia coli* bacterial model. The best variation produced is the addition of 5% TiO_2 to Portland cement with UV irradiation for 60 minutes with an inhibition zone of 7.35 mm.

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