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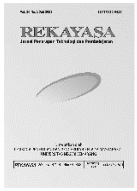

Bactericidal Activity of *Resin-Titanium Dioxide* and Ultraviolet in Killing *Escherichia Coli* Bacteria

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Abstract

Escherichia coli (*E.Coli*) harms the environment in human digestion. *E coli* causes *diarrhoea* if one consumes food or drink that is not clean or exposed to bacteria. This research aims to produce a specimen that combines resin coated with TiO2 and partly ultraviolet light to kill *Escherichia coli* bacteria. The method used in this research is experimental. The activity in this research is mixing TiO2 nanomaterial with resin, then varying the time and exposure to ultraviolet radiation.

Furthermore, observations were made by comparing the number of bacteria found in the two specimens with SEM. Specimens were analyzed using digital image processing. The appearance of the white capsule is the bacteria. The grey bottom is the specimen conclusion in this study was that the TiO2 (*titanium dioxide*) and resin specimens and modified ultraviolet radiation were quite effective in killing *E.coli* which achieved a 91% reduction in the number of bacteria.

Keywords: E.coli, ultraviolet, titanium dioxide, resins, SEM

INTRODUCTION

Escherichia coli (E.Coli) is a type of bacteria that usually lives in the body of the intestine and is found in the intestines of animals. Most types of *E.coli* are harmless and even help maintain a healthy digestive tract. Nevertheless, some can cause diarrhoea if we consume contaminated food or drink (Kumar, 2020). Many people think about associating *E. coli* with food poisoning. These bacteria cause some medical diseases such as pneumonia and urinary tract infections. The facts from the research reveal that up to 95% of urinary tract infections are caused by *E. coli* (Lee et al., 2018).

Some *E. coli* cause sufferers to get a kind of poison called Shiga. This poison damages the lining of the human intestine. The toxin-producing *E. coli* strain is sometimes called STEC

that Clinical STEC serotypes and molecular subtypes isolated from sick humans. STEC infections are associated with extraintestinal pathogens (Fierz et al., 2017). One terrible strain is O157:H7 which can make sufferers very sick. Symptoms experienced by patients include stomach cramps, vomiting, and bloody *diarrhoea*. This type of illness is the leading cause of acute kidney failure in children. Symptoms are more severe and life-threatening, such as kidney failure in adults, bleeding, and seizures resulting from *E. coli.* It causes humans to get emergency assistance if humans have experienced these symptoms.

E.coli contained in food or drink that can enter the digestive tract through the human mouth must be treated immediately. The way to reduce *E coli* bacteria in food or drink is to use antibacterial agents, a group of ingredients that fight pathogenic bacteria. The mode of action of these agents and substances is to kill or reduce the metabolic activity of bacteria their pathogenic effect in the biological environment by gradually minimizing their number. Antibacterial mechanisms of metal nanoparticles include induction of oxidative stress, metal ion mechanism, and non-oxidative mechanisms. It was as well as simultaneous gene mutations in the same bacterial cell for antibacterial resistance to develop (Wang et al., 2017).

The manufacture and use of biomaterials with antibacterial effects in medical treatment plans are overgrowing. Manufacturing products containing antibacterial substances or coatings from materials with antibacterial properties is an exciting topic for research in the medical field. Various materials with antibacterial properties were analyzed in the laboratory and marketed.

Contaminants can cause various health problems, including irritation and toxic effects, surface and systemic infections, allergies and other respiratory or skin diseases (Anderson & Meade, 2014). Results from previous studies have revealed that various microorganisms, including potentially pathogenic species, are detected in the material (Gupta et al., 2019). adaptive strategy to counter metal toxicity to bacteria through the action of metal nanostructures: reactive oxygen species generated on the particle surface result in metal ion release, membrane dysfunction, particle internalization and cytolytic damage, selectivity, binding site and strain specificity of metal nanostructures on cell walls, effect on bacterial growth and viability (Díaz-Visurraga et al., 2011). The effect of TiO2 nanoparticles on microorganisms shows photocatalytic processes in water such as algae, viruses, fungi and bacteria. Aqueous slurry or with aqueous inoculum (sprayed or dropped) in the photocatalytic process of microorganisms. In addition, TiO2 nanoparticles can be used as powders, usually dispersed in aqueous slurries or films/coatings applied to various substrates. Several works have highlighted the very high bactericidal efficiency of various microorganisms.

Inactivation of bacteria by a photocatalytic process involving TiO2 nanoparticles alone or in a transparent layer (varnish) affects the antibacterial. The antibacterial activity of TiO2 was evaluated by UV irradiation in the slurry with physiological water (stirred suspension); and in a drop deposited on a glass plate (Anderson & Meade, 2014). The results confirmed the difference in antibacterial activity between the simple drip inoculum and the inoculum spread under the plastic film, which increased the possibility of contact between TiO2 and bacteria (forced contact) (Viender et al., 2014). In addition, the main effect of suspension properties on photocatalytic disinfection ability is highlighted. Experiments were also carried out on the surface of a transparent layer formulated using TiO2 nanoparticles. The results showed significant antibacterial activity after two h and four h and suggested that increasing the formulation would increase efficiency. Natural bactericidal surfaces

providing a representative structure model on free-form metal implant surfaces remains a scientific challenge (Larrañaga-Altuna et al., 2021).

In this study, researchers focused on using a nanomaterial known as TiO2, which was mixed in a resin to become a composite with antibacterial properties by adding *titanium dioxide* capable of having a short-term effect of an antibacterial agent. Observations will be made by comparing the number of bacteria contained in two different specimens. This application can be used as a coating for covering, wrapping, and protecting food or beverages. TiO2 coated surfaces exhibit a photoactive bactericidal effect with all bacteria used in the ceramics and building industry to produce coated surfaces for placement in microbiologically sensitive environments, such as hospitals and the food industry (Bonetta et al., 2013).

METHOD

The specimens used in this study were resin specimens without a mixture of titanium dioxide and resin specimens with a mixture of titanium dioxide and several treatment variations—antibacterial behaviour when using ultraviolet light, titanium dioxide resin, or combined. Meanwhile, the processing method using digital image analysis was carried out as a further step in research to obtain changes in the percentage value of the number of bacteria present in the specimen.

Resin Manufacturing Procedure

50 mL of polyester resin was formed for one specimen and titanium dioxide in 0.06 g. How to make a specimen by printing it on the moulding that has been provided. Furthermore, *titanium dioxide* wass mixed into the polyester resin. The mixture of resin and titanium dioxide was stirred evenly with an agitation stirrer at a speed of 60 rpm. The resin catalyst was added as much as 10%. Furthermore, stirring was continued to distribute the catalyst in the mixture. The homogeneous mixture was then poured into the mould. If the moulding volume follows the volume of the mixture, then the solid mixture is dried for 24 hours. A resin with a catalyst was used (without getting a transparent/clear and smooth specimen surface).

Procedure for obtaining digital images

a. Scanning Electron Microscope (SEM)

The SEM test was used to get an image of the number of bacterial colonies in the specimen. Specimens were cleaned first so that the surface of the specimen was not covered with dirt. Furthermore, the resin specimen without titanium dioxide treated with *E.Coli* bacteria was treated in a stationary position without being given Ultra Violet C light. SEM immediately tested the results, which was a specimen without UV (0th minute). The specimens were reused and allowed to stand without UV light for 60 minutes and then tested by SEM. The subsequent treatment was that the specimen was then given UV light for 10 minutes and tested using SEM.

The other specimen samples were placed in the cup. An electron beam irradiates the sample with a power of approximately 5 kV. The effect is that secondary electron beams and backscattered electrons were generated, which are detected by the scintillator amplified by the image on the screen after shooting a particular part of the surface—the distance of the object and the required magnification. Furthermore, the resulting image from SEM can be identified. Repetition was carried out on resin specimens with a mixture of 0.06 g of resin with *Titanium dioxide.*

b. Coreldraw 2020

The image is visualized and analyzed with a bacterial image is placed in the form of a box.

The bacterial image was changed to grayscale (8-bit) colour mode. Furthermore, the image of bacteria is divided based on the grayscale level. The images of bacteria are separated based on the grayscale level so that a group of bacterial images is formed based on the grayscale level that was known from knowing the grayscale value of each part of the bacterial image. After processing the digital image, the bacteria and cylinder head area was measured using ImageJ (Bera et al., 2019).

Area Measurement Procedure

Measurement of the surface area using the ImageJ application by processing image results from Coreldraw is cut from the results to be measured using a ruler. Next, the image is scaled. The portion of the bacteria to be measured is selected. After this, the sections were selected, data from the measurement of the area of each section will appear. Finally, the area data was obtained and continued with discussion and analysis.

RESULT AND DISCUSSION

The results and discussion sections contain significant research findings described narratively. The study was carried out with sequential procedures as stated in the method. The test results from this study are in the form of digital images produced by SEM devices that visualize the number of *E. coli* bacteria in the specimens.

This visualization image of the *E.Coli* bacteria will be analyzed further to measure the effectiveness of the resin specimens treated with a mixture of titanium dioxide nanomaterials and modified by exposure to ultraviolet light.

Variations with ultraviolet light were added because irradiation with ultraviolet light was used to kill bacteria. Researchers intend to compare the effectiveness of antibacterial behaviour when using ultraviolet light or titanium dioxide resin or when combined both.

The results of the SEM test visualization for specimens with various treatments can be seen in Table 1. The resin specimens-*titanium oxide* nanomaterials and without *titanium oxide* combined with and without UV light. Specimens were tested without UV at 0 and 60 minutes compared to specimens irradiated with UV for 10 minutes. The visual results of the SEM TEST for specimens with several treatment variations are shown in Table 1. The scale indicates the 2000x SEM magnification on the bars in the figure. The white colour with a capsule-like shape visual represents *E.Coli* bacteria. Observations of SEM morphology on specimens without titanium oxide showed that UV irradiation had a minimal effect on bacteria. Some empty areas appeared, whereas there was no effect on time in specimens without irradiation.

In contrast, the specimen containing TiO2 indicates that the emission due to the presence of a bacterial agent gives a different primary colour in the absence of TiO2. The specimen's colour is lighter (light grey) than without TiO2 (dark grey). The number of *E.Coli* bacteria attached to the specimen was reduced in two conditions: the surface that received ultraviolet light irradiation and the surface of the resin mixed *with titanium dioxide.* It can be seen from the decrease in the thick white lines. The effect of TiO2 is very significant at the time difference of 0 minutes and 60 minutes. It is shown in the number of bacteria, which decreases with the touch between the specimens containing TiO2 and bacteria. The number of bacteria will decrease when added with ultraviolet irradiation, with the observation that almost half of the number of bacteria is reduced from the initial position of 0 minutes. Thus the role of TiO2 dramatically affects the time to reduce the number of bacteria, while the addition of UV irradiation further reduces the number of *E Coli* bacteria. Using SEM after 10 minutes of ultraviolet irradiation, Bacterial cell morphology showed that *E coli* underwent

significant morphological changes. Oval or spherical shape after UV treatment only, round shape with small protrusions, and cell destruction after incubation under UV on TiO2 specimens. Morphological studies of bacterial cells with atomic power microscopy after 60 minutes of irradiation showed that *E coli* 321-5 underwent significant morphological changes. TiO2 film is used as a self-sterilizing surface because of its nature to form reactive oxygen species (ROS) when irradiated with ultraviolet light because bacteria attack and kill them. The increase in the bactericidal activity of TiO2 films can be through the formation of nanopores on the surface (Pleskova et al., 2016). The role of Tio2 modified with other materials to form a *hydrophilic polyurethane/titanium dioxide* (TiO2) complex film after 8 hours, almost all *Escherichia coli* can be killed under visible light irradiation; antimicrobial activity was found to be better than that of pure polyurethane films (Zhang et al., 2008).

Table 1. Visual SEM results with 2000x *E.coli* magnification on different specimen surfaces

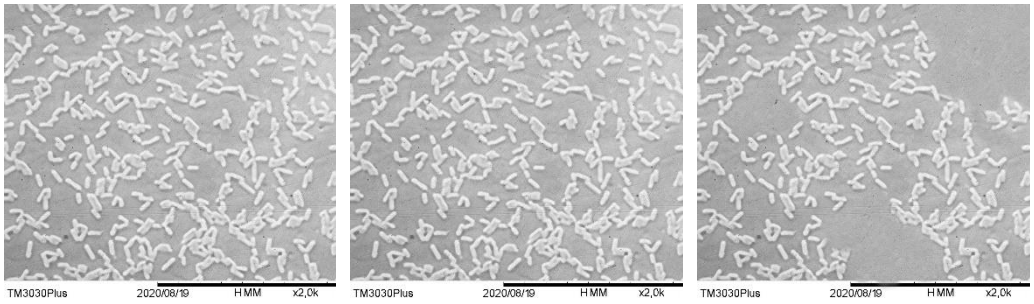
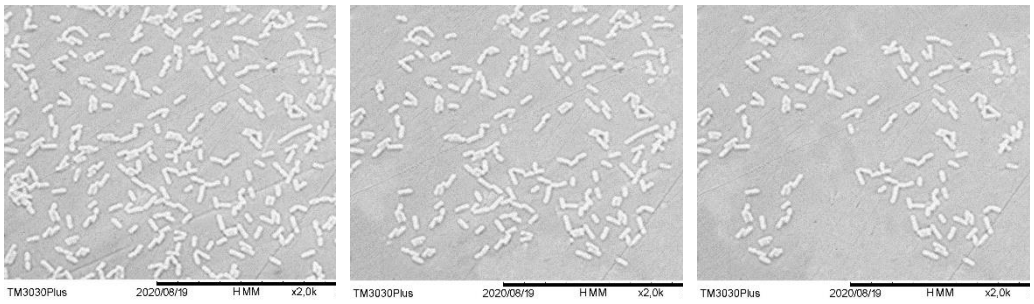
Specimen	**visual *E.coli* pada Spesimen**		
	Without UV	**Without UV for 60 min**	**With UV fo 10 min**
Without TiO_2			
With TiO_2			

Table 2. Digital Image Processing Results Visual SEM 2000x extension of *E.Coli*

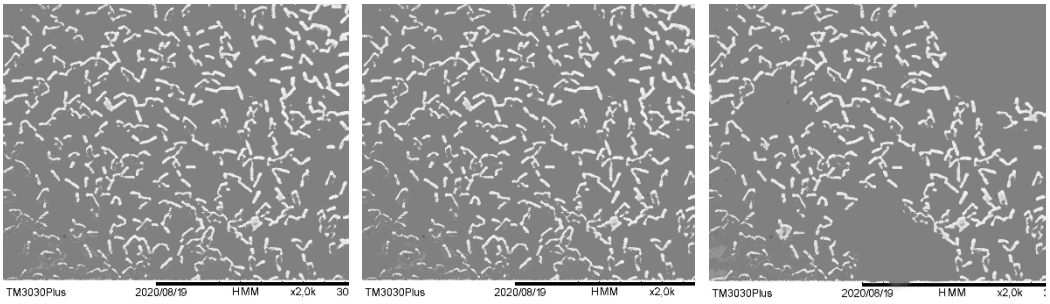
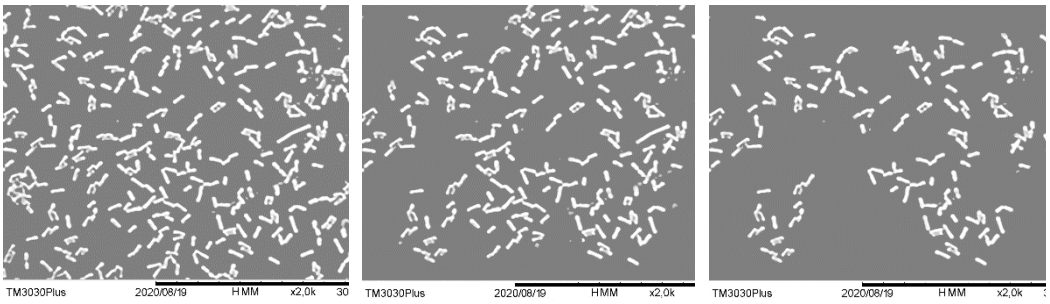
Specimen	**Visual *E.Coli* in Specimen**		
	without UV	**without UV for 60 min**	**with UV for 10 min**
Without TiO_2			
With TiO_2			

Table 3. Results of Calculation of Area Based on Grayscale Level

Specimen	Treatment	Grayscale 12		Grayscale 20		Grayscale 54	
without TiO_2	without UV	800	25%	96	3%	2304	72%
	without UV for 60 min	800	25%	96	3%	2304	72%
	with UV for 10 min	416	13%	160	5%	2624	82%
with TiO2	without UV	608	19%	160	5%	2432	76%
	without UV for 60 min	288	9%	192	6%	2720	85%
	with UV for 10 min	160	5%	128	4%	2912	91%

This method was generally done by separating photos based on their value or grayscale level. Grayscale values were divided into several percentage scale values in white and black. The following are the results of digital image processing. Table 2 shows the results of digital image processing using Corel Draw and ImageJ applications. Visualization images from SEM can be known the extent of each area based on the percentage level of the grayscale value that is getting bigger. The test results analysis compares the surface area values between specimens by recording the grayscale values for each treatment. Analysis of the area calculation based on the grayscale values obtained three grayscale levels, namely grayscale with values of

12, 20, and 54. Grayscale values of 12 and 20 are signs of *E.Coli* bacteria, while the grayscale value of 54 is titanium dioxide resin, a colony of *E.Coli*.

Photochemical activity due to the use of TiO_2 can kill various Gram-negative and Gram-positive bacteria, filamentous and unicellular fungi, algae, protozoa, mammalian viruses, and bacteriophages. The mechanism of the cell wall and cytoplasmic membrane degradation is caused by the production of reactive *oxygen* species such as hydroxyl radicals causing leakage of cellular nuclei then cell lysis and can be followed by complete mineralization of organisms (Foster et al., 2011).

Methodically, the wider the value of the grayscale area with a value of 54, it can be interpreted the fewer the number of *E. coli* bacteria contained in the specimen. Specimens with good performance were recorded in the grayscale value column of 54 with the highest percentage. The percentage presented is the comparison of a grayscale level to the overall area value of the observed specimen. For example, a grayscale value was 12 with an area of 800 mm2 when presented in 25%. The value can be a quarter of the total area of the specimen observed from the total area of 3200 mm2 or 100%.

In contrast, the increase in the area at the grayscale level 54 in two types of specimens, namely those given ultraviolet light irradiation or those using resins with a mixture of titanium dioxide. The largest area at the 54 grayscale levels occurred in specimens that received dual treatment, namely resin specimens using a mixture of titanium dioxide.

In resin specimens that were not mixed with titanium dioxide or in Table 3 written specimens without TiO2, the lowest number of *E Coli* bacteria was visualized in specimens without TiO2, which received ultraviolet light irradiation for 10 minutes. Specimens without TiO2 that received treatment were left at room temperature for 0 minutes and 60 minutes, recording the same *E Coli* bacteria values. Using resin without a mixture of titanium dioxide and prepared at room temperature resulted in *E Coli* bacteria surviving. On the other hand, the resin without a mixture of titanium dioxide but exposed to ultraviolet light reduced the number of surviving *E Coli* bacteria. The number of living bacteria decreased reached 10% of the original number. The surface of the transparent layer formulated using TiO2 nanoparticles showed significant antibacterial activity after 2 hours.

Moreover, reducing the distance between the nanoparticles and bacteria increased the inactivation of *E. coli* by non-photocatalytic effect (direct contact) and photocatalytic disinfection processes (Verdier et al., 2014). The nanocomposite layer with the highest TiO2 load displays antimicrobial activity even without UV light, and the bactericidal effect against Staphylococcus aureus is higher than *Escherichia coli*. An increase can be made in the concentration of nanoparticles, so it is a suitable and inexpensive method to prevent the proliferation of microbes in public places, especially in centres with a higher risk of infection (Díez-Pascual & Díez-Vicente, 2015).

In resin specimens mixed with titanium dioxide, the lowest number of *E Coli* bacteria was visualized in specimens with TiO2, exposed to ultraviolet light for 10 minutes. Meanwhile, the specimens with TiO2 that received treatment were conditioned at room temperature for 60 minutes. It was noted that the number of *E. coli* bacteria was less than that of the specimens with TiO2 that received treatment at 0 minutes. Although the specimens with TiO2 kept at room temperature for 60 minutes recorded a decrease in the number of bacteria, it still could not beat the decrease in the number of bacteria in the TiO2 specimen with ultraviolet irradiation for 10 minutes. Thus the resin with a mixture of TiO2

and irradiated with ultraviolet light showed a reduction in the number of surviving *E coli* bacteria. The decrease in the number of live bacteria when using a mixture of *titanium dioxide* and irradiated with ultraviolet light reached 66% when compared to the resin mixture placed at room temperature for 60 minutes without ultraviolet light, and a 25% decrease when compared to the resin mixture placed at room temperature for 0 minutes without ultraviolet light. According to TiO2, nanoparticle film formation and antimicrobials combine to make promising agents for water disinfection and biomedical applications in the dark and UV illumination (Kim et al., 2019). Ag-TiO2 nanoparticles produced by this type of laser have a broad spectrum of antibacterial effects, including against drug-resistant strains, with several underlying molecular mechanisms and low human cell toxicity so that they can be applied in potential biomedical fields (Korshed et al., 2018).

Resin with a mixture of titanium dioxide can kill bacteria even without being given ultraviolet radiation. If there was want to get a better antibacterial effect, can combine the method of mixing resin with titanium dioxide and the method of ultraviolet irradiation. The *resin-titanium dioxide* mixture specimen could kill bacteria, and the resin with modified ultraviolet light could kill bacteria.

The treatment can be combined in UV before being contacted with TiO2, in the form of a resin-based composite containing 20% TiO2 nanoparticles that continues to provide a significant antibacterial effect against *Escherichia coli* pathogens for two hours after UV treatment (Cai et al., 2013). It is because of the nature of TiO2 itself, which is antibacterial and non-toxic. Mixing TiO2 with resin is also self-cleaning. Applying alternative energy sources in the disinfection of *E. coli* using TiO2 under sunlight can be a possible application for different oxidation processes (Cho et al., 2002). The engineering that can be used for this research is the addition of an antibacterial function to dental resins providing an opportunity to extend their service life by reducing secondary caries caused by bacterial recolonization using nitrogen-doped TiO2 nanoparticles (Zane et al., 2016).

CONCLUSION

The resin with a mixture of titanium dioxide can act as an antibacterial agent, especially *E coli* bacteria. Ultraviolet light can act as a light that can kill *E coli* bacteria. The safety of using titanium dioxide in food-grade materials (dental materials). The change in the strength of the material from materials mixed with *titanium dioxide* needs to be investigated further.

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