Antibacterial Investigation Activity of Titania Anatase technical grade on polypropylene sheet

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Abstract. TiO₂ (titania) has a good potential for photocatalytic activity in degrading the bacteria by attacking the membrane with OH radicals, inhibiting the synthesis of proteins and nucleic acids in bacteria, the OH radicals can be a strong oxidizing agent activated by light irradiation. We investigate the antibacterial activity of Titania anatase in reducing bacterial colonies. Titania powder was deposited on polypropylene by spray method. The formed film was then the heated on the hotplate at 70°C. Antibacterial test was performed using the plate counting method. Principally, the Plate Count Method calculates the number of colonies formed on each plate. Plate Count Method is carried out by dissolving test samples containing bacteria with physiological salt water. Before dissolving, one piece of each sample that has been irradiated under the light for 24 hours was taken. This experiment shows good result of bacteria degradation. Technical grade Titania from Bratachem showed the performance almost the same as nano or pure titania did. The Technical grade titania has potential for large scale application.

1. Introduction
In recent years, semiconductor photocatalyst has received considerable attention since it holds promising potential in both energy and environmental fields. Silver Nanoparticle (Ag), silver oxide (AgO₂), Silicon (Si), Copper oxide (CuO), Zink Oxide (ZnO), Calcium Oxide(CaO), Magnesium Oxide(MgO),TiO₂ are among of the semiconductors that have an antimicrobial ability with their photocatalyst properties. Among various semiconductors, titania (TiO₂) is the most widely used due to its nontoxicity, chemical stability, water insolubility, low price, and good photochemical property. TiO₂ has three phases crystalline, anatase (tetragonal), rutile (tetragonal) which is the most common and natural form of TiO₂ and brookite (orthorhombic), one of the rare forms of TiO₂ phase in nature[1]. Anatase phase is widely used as an electron collecting layer in organic photovoltaic and also used as a catalyst for nanotube and nanoribbon. [2,3,4].

TiO₂ particles have unique properties such as higher stability, safe and a broad spectrum of microorganisms antibiosis [2,3,5]. Antimicrobial properties with respect to TiO₂ crystal structure, shape and size are the main parameters for effectiveness of antimicrobial results [1,6]. the TiO2 in industrial application have been used in photoactive materials, for example antifogging surfaces, self-cleaning, but for medical field applications is still limited and is being developed. The utilization of TiO2 as an antimicrobial has been studied since a few years ago. Choi et al reported that the covering layer of natural oxide commercially pure Ti has antimicrobial behavior, with no significant difference between the non-treated, anodic, and thermal-treated specimens [6]. Anatase band gap higher than
rutile, anatase 3.2 eV and rutile 3.1 eV. The range of anatase bands are higher than rutile. So photocatalytic activity is better than rutile.

TiO$_2$ material can be utilized with ultraviolet due to band gap 3.2 eV and can be easy recombination of the electron-hole pairs[7]. Bactericidal effect of TiO$_2$ under UV-light is very useful to disinfect contaminated surfaces of dental implants as reported by Suketa et al [8]. The author uses a plasma source ion implantation to deposit the TiO$_2$ layer to titanium metal. According to Livia et al, the application of TiO$_2$ coating as antibacterial, should use the activation mechanism of photon with sufficient energy and reach the semiconductor surface to activate the catalytic process. The activation energy problems can be solved by doping the TiO$_2$, photocatalyst development shows high reactivity under visible light ($\lambda > 400$ nm) [9]. The advantage of photocatalytic utilization on TiO$_2$ surface is without electricity or chemical reagents, because light, oxygen and water are just the materials needed. TiO$_2$ surface is nontoxic and does not cause environmental pollution [9]. These characteristics make TiO$_2$ material an option for medical applications in the future. Here, in this experiments we try to explore the ability of technical grade TiO$_2$ for bacteria degradation with a simple procedure by plate counts method.

2. Experimental Method

2.1. Material

Technical Grade Titania (TiO$_2$) anatase, PVA (Polyvinyl Alcohol) purchased from Bratachem, Indonesia. Aquades from Sakura Indonesia, and Polypropylene sheet. For bacteria we use bacteria gram negative and positive i.e Staphylococcus Aureus and Escherichia Coli due to their large availability in nature.

2.2. Experimental

The sample preparation begins with a solution consist of polyvinyl alcohol with a similar variation and TiO$_2$ powder were dissolved in 50 ml of deionized water in warm conditions of 70°C. The solution was stirred for 60 minutes, then embed into substrate by spraying on polypropylene sheet. We use coating method from previous research [10]. The PC sheets covered by TiO$_2$ particles were then pressed down at 110°C for 4 minutes, Pressing and heating process were carried out using a modified laminate equipment. The samples were then rinsed by aquades to wash out the weakly bound particles. Finally the TiO2/PC sheets were dried using a warm air flow.

2.3. Antibacterial testing using the simple plate count method

Simple Plate Count Method principally counts the number of colonies formed into each plate. Plate Count Method was used by dissolving the sample containing the bacteria with saline water. Before dissolving, one piece of each samples which have been irradiated under the light for 24 hours was taken. Sample was then put into 10 ml physiological saline water and stirred with vortex equipment (dilution 10 times). Then 1 ml of this solution was taken and mixed at 9 ml physiological saline solution and stirred with a vortex (dilution 100 times). This activity was repeated until 1 million times. Pour the solution into two different plates and mixed with nutrient as medium of growing bacteria. After 24-48 hours, bacteria plate was taken out and manually the number of colonies was counted. Irradiation on anatase TiO$_2$ as antibacterial was performed using UV radiation C. UV-C radiation has shorter wavelength and higher intensity than UV-A or UV-B. Tertanum reported that photocatalytic activity increased significantly under irradiation of UV-C. In this experiment, we use Halogen lamp to replace sun light at night since Halogen has spectrum near UV-infrared [11]. The illustration of dilution showed on figure 1 below, and the photograph of irradiation sample showed in figure 2.
3. Result and discussion

3.1. Antibacterial activity from TiO$_2$

We do two steps in this experiment. Material coating and bacterial testing. After coating, proceed with the test by simple plate count method. For material coating process we did in interdisciplinary physics laboratory, Department of physics ITB. The bacterial testing process was done in microbiology laboratory of biosciences and biotechnology research center ITB. We got the bacteria test result shown in table 1. Sun or ultraviolet light that is used to degrade bacteria, has many uses when converge with certain materials as shown in figure 3a. The possible scheme for bacterial degradation with TiO2 is shown in Figure 3b.
Figure 3 a. Irradiation illustration from Sun (ultraviolet)

Figure 3 b. Schematic of Bacteria degradation by TiO$_2$

Figure 3 a, is illustration of irradiation from sunlight into any compound around the environment. The light irradiation of sun (ultraviolet) can be used in various benefits for example degradation, deodorization, sterilization. Figure 3b. showed the schematic of bacteria degradation by irradiation from sun and TiO$_2$. The effects of TiO$_2$ are associated with OH radicals and reactive oxygen species (ROS) generated from the photocatalytic process where cell membranes are the primary attacked target. Then lipid peroxidation which is located at the ends of cell membrane eventually become lysis and damaged. In other report, Sunada et al proposed a three-step mechanism for photokilling of bacteria on irradiated TiO$_2$-surfaces. First, TiO2 attack of cell walls by reactive oxygen species second, disordering of the inner cytoplasmic membrane and killing of the cell third, decomposition of the toxic ingredients of bacteria [12]. The antibacterial properties of TiO$_2$ are reported being non-toxic and widely accumulated in many areas of research and currently attracted the attention to be developed as commercial antibacterial. Antibacterial activity was quantitatively observed using plate count method.

The focus of antibacterial test, is to observe the anatase titania activity in degrading bacteria around human that cause infection such as E.Coli and S.Aureus. We know that S.Aureus and E.Coli live in temperature ranging 4°C-48°C and pH 4.5-9.3 [13]. Since TiO$_2$ was irradiated by UV-Light, It generated electron- hole pair that caused redox reaction on the surface of TiO$_2$. As the effect of electron excitation from valence band to conduction band, the negatively charged (electron) and oxygen are banded to become O$_2^-$, whereas positively charged (hole) and water produced radicalhidroxil [13]. Radicalhidroxil caused the bacteria oxidized, cytoplasmic eukaryotic and prokaryotic cells reduced, cell damaged and eventually made the bacteria dead[13].
The experimental process using the plate count method was shown in figure 4 below. We took the photograph of the random sample containing control sample and antibacterial sample.

![Figure 4. Photograph of control sample and bacteria agent](image)

Experiment data of bacteria growth inhibition was shown at table 1 below.

| Treatment | S.Aureus (colony) [CFU/ml] | E.Coli (Colony) [CFU/ml] |
|-----------|---------------------------|--------------------------|
| Plate     | 1 x 10^6                  | 2 x 10^6                 |
| control   | 220 x 10^6                | 212 x 10^6               |
| Sample    | 7 x 10^6                  | -                        |
|           | 276 x 10^6                | 263 x 10^6               |
|           | 8 x 10^6                  | -                        |

CFU/ml : Colon Forming Unit/ml

After incubation for 24-48 hours in dark room, we collect data growth inhibition of bacteria, showed at Table 1. The control plate signify a high bacteria growth. This proved that the bacteria can grow in the experiment environment after irradiation and incubation. But, in the second sample contain the bacteria after dilution of the coated sample. The data indicate bacterial growth obstructed after irradiation and incubation. It is obviously observed that technical grade of anatase TiO_2 is effective in inhibiting the growth of bacteria. The difference in the photocatalytic activity of different structures is closely related to the surface area and particle size of the photocatalyst. Particles bulk structure anatase is more reactive than rutile and brookite. This is due to higher bandgap owned by Anatase compared to Rutile bandgap (3.2 eV : 3.1 eV). Therefore, anatase has a better photocatalytic activity [14]. In this study we clearly find that technical grade TiO_2 anatase has good potential for photocatalytic process, especially for bacteria degradation.

4. Conclusion
In this study, using the Plate Count Method, it is confirm that TiO_2 anatase has antibacterial properties on ecoli and aureus sample. The temperature and ph use in antibacterial particles and testing does not exceed the optimum temperature of the bacteria. It is clearly evident that the effect of bacteria degradation originate from photocatalyst activity of Anatase Titania. From this preliminary research we can develop for the next application.
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