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Antibacterial Test of the $CaTiO_3$ Compound Obtained through Molten Salt Method

Siti Qurotul Aeni[1,a], Anik Maunatin[1,b], and Anton Prasetyo[1,c*]

[1]Department of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jalan Gajayana 50 Malang, 65144 Indonesia

[a]qurotulaeni8121@gmail.com, [b]anik.maunatin@uin-malang.ac.id, [c*]anton@kim.uin-malang.ac.id

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Abstract. Microbial resistance has become a threat that causes thousands of deaths yearly; therefore, efforts are required to address this problem. One of the promising methods used to inhibit bacterial growth is photocatalyst technology. In this research, $CaTiO_3$ compounds was synthesized using the molten salt method and tested for antibacterial activity in both UV-unexposed and UV-exposed conditions on *Staphylococcus aureus* bacteria. The diffractogram showed that the $CaTiO_3$ compound was successfully synthesized without impurities that indicated by the characteristic peaks at 2θ (°) = 23.29; 33.18; 47.52; 59.33; 69.48; 79.17. The micrograph results showed that the $CaTiO_3$ compound had a regular polyhedral shape and was agglomerated with particle sizes in the 0.2941 ± 0.0144 μm range. The UV-Vis DRS spectra showed that the $CaTiO_3$ compound had a bandgap energy of 3.48 eV (315 nm). In the antibacterial activity test results under UV irradiation, the growth of *Staphylococcus aureus* decreased by 3.95, 0.91, and 1.45 CFU/m

Introduction

According to the World Health Organization (WHO), microbial resistance represents a global health threat. Thousands of people die each year due to bacterial infections. Some of the most deadly bacteria include *Escherichia coli, Mycobacterium tuberculosis, Salmonella, Staphylococcus, and Bacillus anthracis*. These bacteria infect humans through water, food, and livestock [1]. One potential method for inhibiting bacterial growth involves photochemical reactions and photocatalysis. Both methods degrade environmental substances using ultraviolet (UV) radiation and other forms of light. Photocatalysis is used as an antibacterial agent because reactive oxygen species (ROS) will react with cell membranes and then enter the bacterium, causing membrane disruption and leakage of internal contents, ultimately turning off DNA and proteins within the bacteria [1]. Perovskite structured compounds have been reported to possess photocatalytic properties. The general formula of perovskite compounds is ABO_3, with the A and B positions occupied by metal cations and O for oxygen anions. The A cation is a monovalent, divalent, or trivalent metal ion such as La, Pr, Nd, or K, Na, Li, Ba, Ca. Meanwhile, the B cation is a transition metal ion such as Ti, Ni, Fe, Co, and Mn, located in the center of the oxygen anion octahedron [2].

One of the potential applications of perovskite photocatalyst compounds is as an antibacterial material. Zhang et al. have reported the utilization of the photocatalytic properties of Fe-doped $SrTiO_3$ compounds to inhibit the growth of *Escherichia coli* bacteria at a concentration of 10^5 CFU/mL within 15 minutes [3]. $CaTiO_3$ is a semiconductor photocatalyst that belongs to the perovskite compounds, consisting of Ca (II) and Ti (IV) oxides combined with the $[TiO_3]^{2-}$ ion. The Ca^{2+} ion has a coordination number of 12, located in the center of the unit cell, and each Ti (IV) core has a coordination number of 6 [2]. $CaTiO_3$ has a band gap energy of 3-3.5 eV. The photocatalytic properties of $CaTiO_3$ have been reported and can be utilized for the degradation of organic pollutants, water splitting into H_2 and O_2, CO_2 reduction, and other applications [4]. In addition, Chong et al. have reported that $CaTiO_3$ is capable of degrading methylene blue-colored waste within 60 minutes by 96.6%. However, the utilization of the photocatalyst compound $CaTiO_3$ as an antibacterial agent still limited. Therefore the study about the application photocatalyst $CaTiO_3$ as antibacterial is important

Photocatalytic activity shows that the morphology, shape, and size of particles greatly influence photocatalytic activity. The effect of the particle morphology of $CaTiO_3$ on photocatalytic properties has been reported by Dong et al. stating that $CaTiO_3$ with dendritic morphology has higher photocatalytic activity compared to 'cross' cubic and prism-shaped structures. This is because the smaller the crystal size, the greater the electron transfer distance in the dendritic morphology, resulting in increased light capture. The effect of morphology on antibacterial activity has also been reported by several parties. Listiani et al. tested the antibacterial activity of TiO_2 products and found that the smaller the nanoparticle size, the better the inhibition of bacterial size. It's indicated that the morphology particle influence to antibacterial properties of photocatalyst material.

One synthesis method that has the potential to produce unique particles with uniform size is the molten salt method. The molten salt method is a synthesis method that using ionic salts as a liquid solvent at high temperatures above their melting point [8]. This method is very environmentally friendly and simple. It is often used to synthesize metal oxides, yielding products of high purity, unique morphology, and reducing agglomeration [9]. Several researchers have reported the synthesis of $CaTiO_3$ compounds using the molten salt method. Yoshida et al. obtained a regular polyhedral morphology with high photocatalytic activity from synthesizing $CaTiO_3$ using NaCl molten salt. Additionally, research conducted by Novianti et al. synthezed Fe doped $CaTiO_3$ products with polyhedral morphology which synthesized by molten salt method that used NaCl salt at 900°C for 8 hours. In this study, The $CaTiO_3$ was synthesized by molten salt method and then evaluated the antibacterial properties in inhibiting the growth of *Staphylococcus aureus*.

Material and Methods

Materials

$CaCO_3$ (Aldrich, 99,9% powder), TiO_2 (Aldrich, 99,9% powder), NaCl (Aldrich, 99% powder), KCl (Aldrich, 99% powder), $AgNO_3$ (Aldrich 2,3%), aseton, akuades, blank disk (kontrol), nutrient broth (NB), nutrient agar (NA), asam sitrat (1:1), alumunium foil, cotton.

Synthesis of $CaTiO_3$ via the salt melt method

Precursors were used based stoichiometric calculation and ratio mole product and salt (NaCl/KCl) is 1:7:7. Precursors and salt were homogenized by grinding them in an agate mortar for one hour. The mixture was then calcined in a furnace at temperatures of 750°C and 820°C for six hours. Then, the sample product was washed with warm distilled water to remove any remaining salt. The amount of residual salt was analyzed by adding silver nitrate solution to the filtrate. Finally, the sample product was dried in an oven at 80°C for two hours.

Characterization

The characterization techniques used in this research are: (a) XRD (X-Ray Diffraction) (Rigaku Miniflex with Cu as the X-ray source) technique for determining the crystal structure and phase composition, and the measurements were conducted at 2θ: 10° to 90°, (b) scanning electron microscopy (SEM) (Hitachi flexsem 1000) was conducted to get the morphology particle image, and its measurement was used magnifications ranging from 3,000 to 20,000 times (c) ultraviolet-visible diffuse reflectance spectroscopy (UV-Vis Spectroscopy) (Thermo Scientific Evoluation 220) measurements was conduct to determine reflectance spectrum and the calculate it to obtain bandgap energy.

Antibacterial Activity Test

Bacterial and Inoculum Preparation

Staphylococcus aureus was cultured on nutrient agar (Merck, Germany) and incubated at 37°C for 24 hours. The inoculum was prepared by transferring multiple colonies of *Staphylococcus aureus* into the nutrient broth (Merck, Germany) and incubating it at 30°C with shaking at 100 rpm for 18 hours. The turbidity of the inoculum was adjusted to an optical density (OD) of 0.1 at 600 nm [11].

Antibacterial Activity

The antibacterial activity test was performed by adding 10 mL of inoculum to 100 mL of nutrient broth containing different masses of the $CaTiO_3$ compound, specifically 50, 100, and 150 mg. These mixtures were exposed to UV light for 2 hours. Control tests were also conducted without the addition of $CaTiO_3$ and UV light. The viability of *Staphylococcus aureus* was observed following the test.

Viability of Staphylococcus aureus

The viability of *Staphylococcus aureus* was determined using the pour plate method. After the test, 1 mL of the media was taken and diluted in 9 mL of sterile 0.85% NaCl, with serial dilutions ranging from 10^{-1} to 10^{-7}. A 0.1 mL sample from each dilution was plated in duplicate on petri dishes, followed by the addition of nutrient agar. The plates were incubated at 37°C for 24 hours, after which the colonies were counted. Cell viability was expressed as colony-forming units (CFU) per milliliter [12].

Result and Discussion

The diffractogram of the $CaTiO_3$ product is shown in Figure 1 and was compared to the standard pattern from the Inorganic Crystal Structure Database (ICSD) No. 71915, which corresponds to an orthorhombic crystal system with the space group Pbnm. The comparison showed that the sample's pattern matches the standard that indicated the $CaTiO_3$ was successfully synthesized. The presence of characteristic $CaTiO_3$ peaks at 2θ = 23.29°, 33.18°, 47.52°, 59.33°, 69.48°, and 79.17°. There are no additional peaks that indicate the impurities compound did not form. It indicates that the synthesis conditions, including the type of salt, molar ratio of product to salt, and temperature, were suitable for the reaction. However, a slightly broad peak in the 2θ range of 20-25° suggests the presence of an amorphous phase in the product.

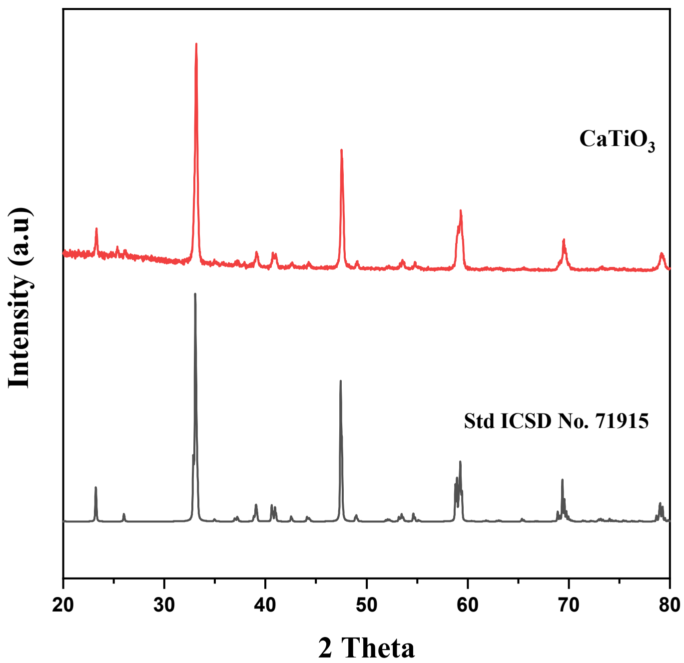

Fig. 1. Diffractogram of $CaTiO_3$

The SEM image is shown in Figure 2, and it can be seen that the morphology of $CaTiO_3$ particle is irregular polyhedra. Its morphology is similar to the findings of Novianti et al. who synthesized Fe-doped $CaTiO_3$ via the molten salt method that used NaCl Salt with a synthesis temperature of

900°C for 8 hours. The result of particle size distribution analysis using Image-J software is shown in Figure 3. The particles are relatively large, with an area of 0.2941 ± 0.0144 µm. The irregular shapes and large grain sizes also indicate the agglomeration due to the high-temperature calcination synthesis. The calcination at high temperatures increases total surface energy and causes particles to clump together to reduce overall surface energy [9].

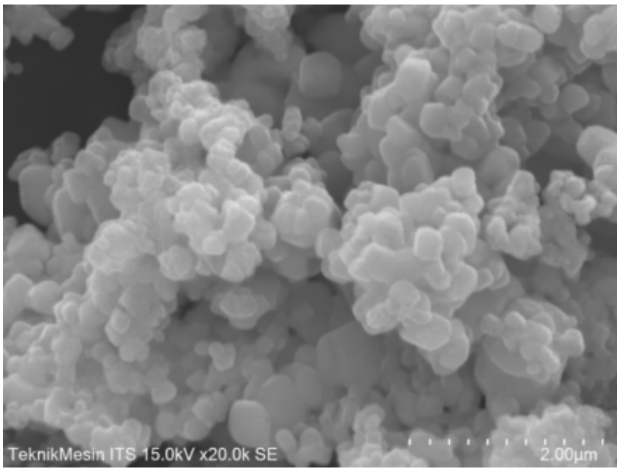

Fig. 2. Micrograph of $CaTiO_3$ compound with 20,000x magnification

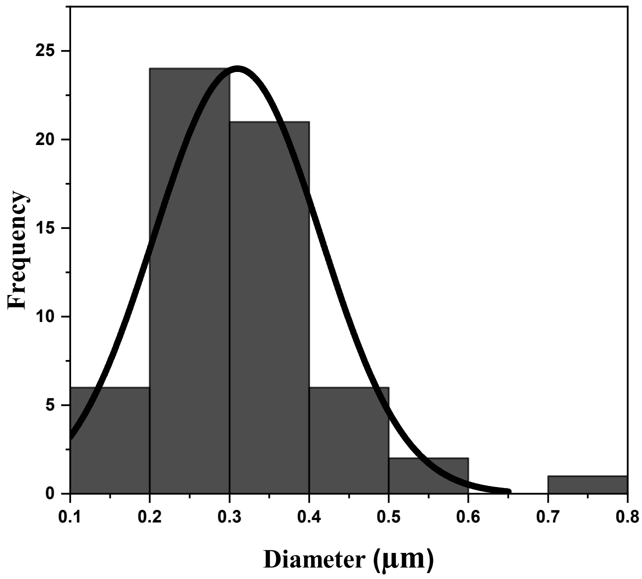

Fig. 3. SEM particle distribution of $CaTiO_3$ compound

The reflectance spectrum of $CaTiO_3$ compound is shown in Figure 4 and indicates the light absorption ability in the 200–400 nm wavelength range. The reflectance spectrum was analyzed using the Kubelka-Munk equation with an indirect-gap approach. The Tauc plot is shown in Figure 5, and it can be seen that the bandgap energy of $CaTiO_3$ was found to be approximately 3.48 eV (315 nm) and is similar to the previous reported by Yang et al. This bandgap energy results from electronic transitions involving electrons from the O 2p orbitals in the valence band to the Ti 3d orbitals in the conduction band [9].

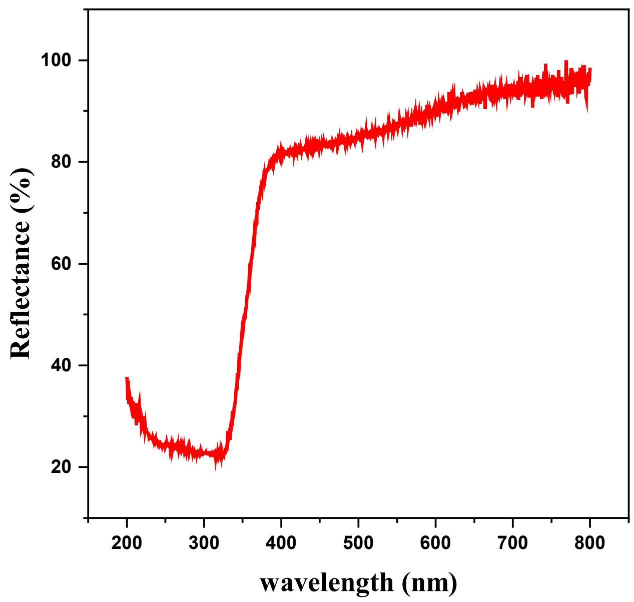

Fig. 4. Plot of data (%) reflectance with wavelength (nm) of $CaTiO_3$ compound

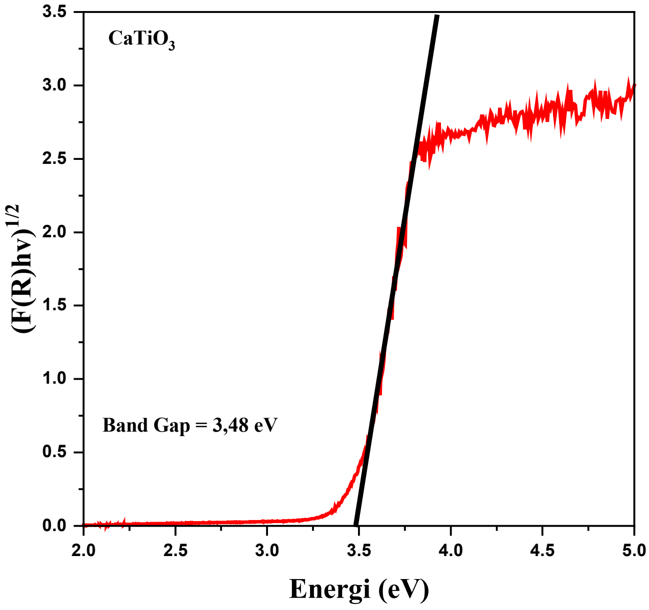

Fig. 5. Tauc plot of band gap energy of $CaTiO_3$ compound

The results of the antibacterial activity test of $CaTiO_3$ against *Staphylococcus aureus* are presented in Figure 6, which shows the highest bacterial growth in open-air conditions with a value of 41.45 x 10^7 CFU/mL. The standard deviation observed in open-air conditions was very high at 40.38, leading to significant fluctuations. This occurred because bacterial growth was unhindered and difficult to control, resulting in rapid and uncontrolled proliferation. In dark (control) conditions, bacterial growth increased compared to UV-irradiated (control) conditions, as the dark environment is highly favorable for bacteria that are not directly exposed to UV light. Conversely, UV irradiation can cause damage to bacteria and microorganisms, thus reducing growth. Additionally, Figure 6 shows that bacterial growth with the addition of $CaTiO_3$, whether exposed to UV light or not, experienced a decline. In the absence of light, there was a reduction in *Staphylococcus aureus* growth because $CaTiO_3$ can inhibit bacterial growth, as $CaTiO_3$ compounds possess antibacterial properties. Heavy metal compounds are toxic to bacteria because heavy metal ions have positive charges that easily react with the negative charges of bacterial proteins, leading to denaturation of bacterial cells [15]. Figure 6 also shows that with the addition of UV-irradiated $CaTiO_3$, bacterial growth decreased due to the photocatalytic processes involved in antibacterial activity. The mechanism that occurs during the irradiation of $CaTiO_3$ with UV light generates Reactive Oxygen Species (ROS), which then react with the bacteria. These ROS can increase stress on the cells, aiming to damage cellular components such as proteins, ribosomes, DNA/RNA, peptidoglycan, etc. [16]. Proses antibakteri fotokatalitik melibatkan interaksi antara bakteri dan reactive oxygen species (ROS), yang meliputi oksigen singlet ($^1O_2$), radikal hidroksil (•OH), dan anion radikal superoksida ($•O_2^-$). The photocatalytic antibacterial process involves the interaction between bacteria and reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), hydroxyl radicals (•OH), and superoxide anion radicals ($•O_2^-$). These ROS interact with the bacterial cell membrane, penetrate it, and cause damage to the membrane. As a result, the contents of the bacteria leak out, while the bacterial DNA and proteins are deactivated [1].

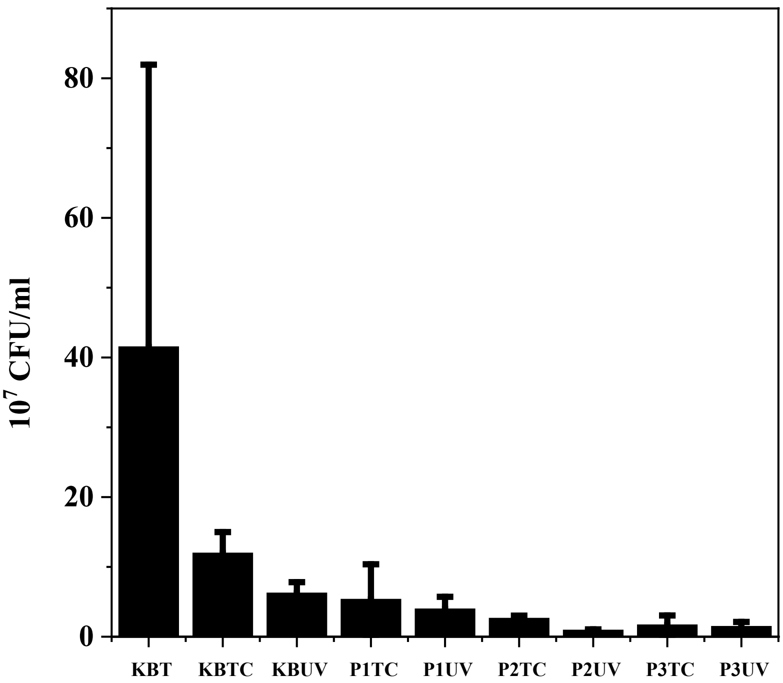

Fig. 6. Antibacterial test of $CaTiO_3$ compound on *Staphylococcus aureus* bacteria open bacterial control (KBT); bacterial control without light/dark (KBTC); bacterial control with UV (KBUV); $CaTiO_3$ 50 mg without light/dark (P1TC); $CaTiO_3$ 50 mg with UV (P1UV); $CaTiO_3$ 100 mg without light/dark (P2TC); $CaTiO_3$ 100 mg with UV (P2UV); $CaTiO_3$ 150 mg without light/dark (P3TC); $CaTiO_3$ 150 mg with UV (P3UV)

The antibacterial testing involved three concentrations of $CaTiO_3$: 50 mg, 100 mg, and 150 mg. Under dark conditions, bacterial growth decreased as the $CaTiO_3$ concentration increased since higher concentrations result in more heavy metal ions, which can damage bacterial cells. Similarly, under UV irradiation, bacterial growth declined further as the concentration of $CaTiO_3$ increased due to higher concentration producing more Reactive Oxygen Species (ROS) and causing intensively damaged bacterial cell structures and components.

Conclusions

The compound $CaTiO_3$ was successfully synthesized using a molten salt method with NaCl-KCl salts. The morphology of the obtained $CaTiO_3$ particles was polyhedral with a bandgap energy of 3.48 eV (315 nm). Antibacterial testing showed that the $CaTiO_3$ compound could inhibit the growth of *Staphylococcus aureus* bacteria.

References

[1] Zhou, Z., Li, B., Liu, X., Li, Z., Zhu, S., Liang, Y., Cui, Z., & Wu, S. (2021). Recent Progress in Photocatalytic Antibacterial. *ACS Applied Bio Materials*, *4*(5), 3909–3936. https://doi.org/10.1021/acsabm.0c01335

[2] Ali, R., & Yashima, M. (2005). Space group and crystal structure of the Perovskite $CaTiO_3$ from 296 to 1720 K. *Journal of Solid State Chemistry*, *178*(9), 2867–2872. https://doi.org/10.1016/j.jssc.2005.06.027

[3] Zhang, L., Tan, P. Y., Chow, C. L., Lim, C. K., Tan, O. K., Tse, M. S., & Sze, C. C. (2014). Antibacterial activities of mechanochemically synthesized perovskite strontium titanate ferrite metal oxide. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *456*, 169–175. https://doi.org/10.1016/j.colsurfa.2014.05.032

[4] Passi, M., & Pal, B. (2021). A review on $CaTiO_3$ photocatalyst: Activity enhancement methods and photocatalytic applications. *Powder Technology*, *388*, 274–304. https://doi.org/10.1016/j.powtec.2021.04.056

[5] Han, C., Liu, J., Yang, W., Wu, Q., Yang, H., & Xue, X. (2017). Photocatalytic activity of CaTiO3 synthesized by solid state, sol–gel and hydrothermal methods. *Journal of Sol-Gel Science and Technology*, *81*(3), 806–813. https://doi.org/10.1007/s10971-016-4261-3

[6] Dong, W., Zhao, G., Bao, Q., & Gu, X. (2016). Effects of morphologies on the photocatalytic properties of CaTiO3 nano/microstructures. *Journal of the Ceramic Society of Japan*, 124(4), 475–479

[7] Listiani, D., Sapar, A., & Aritonang, A. B. (2019). Sintesis TiO_2-Kaolin Dan Uji Aktivitas Fotokatalisis Untuk Antibakteri *Escherichia coli* dan *Staphylococcus aureus*. *Indonesian Journal of Pure and Applied Chemistry*, 2(3), 130.

[8] Jing Fu, YudongHou, Xuepeng Liu, MupengZheng, Mankang Zhu. (2020). Construction Strategy of Ferroelectrics by Molten Salt Method and Its Application in Energy Field. *Journal of Materials Chemistry C*

[9] Novianti, D. R., Haikal, F., Rouf, U. A., Hardian, A., & Prasetyo, A. (2022). Synthesis and Characterization of Fe-Doped $CaTiO_3$ Polyhedra Prepared by Molten NaCl Salt. *Science and Technology Indonesia*, *7*(1), 17–21. https://doi.org/10.26554/sti.2022.7.1.17-21

[10] Yoshida, H., Zhang, L., Sato, M., Morikawa, T., Kajino, T., Sekito, T., Matsumoto, S., & Hirata, H. (2015). Calcium titanate photocatalyst prepared by a flux method for reduction of carbon dioxide with water. *Catalysis Today*, 251, 132–139.

[11] Rahmawati, R. (2014). Antibacterial activity test of ethanol extract from dragon scale leaves (*Drymoglossum piloselloides* (L.) Presl) and binahong (*Anredera cordifolia* (Ten.) Steenis) against *Streptococcus mutans* bacteria (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).

[12] Retnowati, P. A., & Kusnadi, J. (2014). Production of Probiotic Date Juice (*Phoenix dactylifera*) Using *Lactobacillus casei* and *Lactobacillus plantarum* Isolates [In Press April 2014]. *Jurnal Pangan dan Agroindustri, 2*(2), 70-81.

[13] Gupta, S. K., & Mao, Y. (2021). A review on molten salt synthesis of metal oxide nanomaterials: Status, opportunity, and challenge. *Progress in Materials Science*, 117, 100734.

[14] Yang, H., Han, C., dan Xue, X. 2014. Photocatalytic Activity of Fe-Doped $CaTiO_3$ under UV–Visible Light. *Journal of Environmental Sciences*, 26(7): 1489-1495

[15] Yang, H., Liu, D., & Xue, X. (2012). Antibacterial analyse of perovskite. *Journal of Shanghai Jiaotong University (Science)*, *17*(3), 337–340. https://doi.org/10.1007/s12204-012-1282-0

[16] Regmi, C., Joshi, B., Ray, S. K., Gyawali, G., & Pandey, R. P. (2018). Understanding Mechanism of Photocatalytic Microbial Decontamination of Environmental Wastewater. *Frontiers in Chemistry*, *6*, 33. https://doi.org/10.3389/fchem.2018.00033

[17] Gu, Y., Rabe, K., Bousquet, E., Gopalan, V., & Chen, L.-Q. (2012). Phenomenological thermodynamic potential for CaTiO3 single crystals. *Physical Review B*, 85(6), 064117.

[18] Ganguly, P., Byrne, C., Breen, A., & Pillai, S. C. (2018). Antimicrobial activity of photocatalysts: Fundamentals, mechanisms, kinetics and recent advances. *Applied Catalysis B: Environmental*, 225, 51–7

[19] Kumar, R., Anandan, S., Hembram, K., & Narasinga Rao, T. (2014). Efficient ZnO-Based Visible-Light-Driven Photocatalyst for Antibacterial Applications. ACS Applied Materials & Interfaces, 6(15)

[20] Zhuang, J., Tian, Q., Lin, S., Yang, W., Chen, L., & Liu, P. (2014). Precursor morphologycontrolled formation of perovskites $CaTiO_3$ and their photo-activity for As(III) removal. *Applied Catalysis B: Environmental*, 156–157, 108–115