

Article

Facile Fabrication of Oxygen-Defective ZnO Nanoplates for Enhanced Photocatalytic Degradation of Methylene Blue and In Vitro Antibacterial Activity

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Abstract: In this study, we examined whether catalysts with many defects have excellent photoactivity. We prepared ZnO nanoplates with varying degrees of defects in a short time of 4 h by varying the crystal growth temperature at 50, 100, 150, and 200 °C under a strong alkali NaOH atmosphere of 4.0 M. During high-temperature preparation of ZnO, crystal defects were reduced and crystallinity was further increased. In crystallized systems over 100 °C, rhombic nanoplates were used to control particle shape and induce growth in only two axes. The PL, Raman, and XPS analyses confirmed the presence of strong oxygen vacancies in all ZnO nanoplates, and the vacancies decreased with increasing crystallization temperatures. Methylene blue (MB) dye was initially fixed at 50 mg/L with a peak decrease in absorption at 600–700 nm, confirming its decomposition over time. For the 5 h reaction, the MB removal concentration follows the following order: ZnO-50 < ZnO-100 < ZnO-150 < ZnO-200. The study confirms that ZnO-200 nanoplates with fewer oxygen vacancies decompose MB more quickly. ZnO-200 nanoplates synthesized at 200 °C provided the best sterilization performance when tested against gram-positives and gram-negatives, *Escherichia coli* and *Staphylococcus aureus*, respectively. ZnO-200 nanoplates after 3 h showed a high sterilization performance of 96.95% (86.67% in a dark room) for *staphylococcus aureus* and 95.82% (74.66% in a dark room) for *Escherichia coli* when irradiated with light. Particularly noteworthy in this study is that $\cdot\text{OH}$ and $\cdot\text{O}^{2-}$ radicals are generated more strongly in ZnO-200 than in ZnO-50 nanoplates. These results show that too-strong oxygen vacancies rather inhibit the antibacterial performance, and that the virtue of moderation also exists in the catalytic activity.

Keywords: degree of defects; crystallinity; ZnO nanoplate; oxygen deficiency; antibacterial performance; photodegradation of dye



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1. Introduction

As people around the world are increasingly interested in antibacterial materials due to the recent outbreak of the COVID-19 pandemic, the excellent antibacterial activity of nanosized metal oxides is being intensively revealed. The antibacterial activities of metal oxide nanoparticles are various depending on particle size [1], surface area [2], crystallinity [3], capping/stabilizing agent [4], particle shape [5], concentration/capacity used [6], the pH of the solution [7], and the characteristics of the microorganisms [8]. In particular, smaller nanoparticles and more suitable crystalline shapes can easily penetrate through the pores of bacteria, so they can exhibit more effective antibacterial properties [9,10]. Therefore, developing nano antibacterial materials that fit the optimized parameters is very important as a strategy to prepare for new pathogens in near future.

In particular, among many metal oxide nanomaterials, ZnO is the most widely used and promising antibacterial material [11,12].

Dyes containing methylene blue (MB) dye can cause serious health and environmental hazards, and they are toxic, carcinogenic, and non-biodegradable [13,14]. A number of health risks are associated with MB, including respiratory distress, abdominal disorders, blindness, and digestive and mental disorders. In aquatic organisms, methylene blue induces oxidative stress and damages DNA, causing growth inhibition, developmental abnormalities, and even death [15]. For aesthetical and toxicological reasons, there is a significant threat from MB discharge into the environment [16]. In addition, it reduces light penetration as well as being a toxic source of food for organisms. As a result, metal oxide nanomaterials such as ZnO can also be used for MB dye photodegradation studies in current research for making water drinkable.

ZnO nanoplates are II-VI semiconductors with an equivalent band gap energy of 3.3 eV—similar to TiO_2 [17], but it has lower production cost and higher biostability than TiO_2 [18]. Because of its unique optical properties, which can withstand high temperature and high power operation while having a large electric field, it is used as an interesting material in biology, medicine, and engineering such as solar cells, gas sensors, chemical sensors, biosensors, LEDs, and photodetectors. The material ZnO is an extremely versatile semiconductor material that has a wide range of applications in catalysis. Several reactions have been catalyzed by ZnO, including photocatalytic degradation of organic pollutants, CO_2 reduction, and hydrogen production. A variety of organic compounds, including pharmaceuticals and fine chemicals, can be synthesized with ZnO as a catalyst. Furthermore, ZnO can be used to treat wastewater by degrading various pollutants. Due to its unique physicochemical properties, ZnO is attractive for catalytic applications due to its high surface area and efficient charge transfer [19–21].

Nanostructured ZnO can have various shapes and characteristics, and antibacterial and optical activities are different depending on the shape and size [22]. In general, gram-negative bacteria are less sensitive to metal oxide nanoparticles than gram-positive bacteria in antibacterial evaluation. This is due to the characteristics of the cell wall structure of gram-negative bacteria. Unlike gram-positive bacteria, the cell wall of gram-negative bacteria has an additional outer membrane containing lipopolysaccharide (LPS). LPS enhances the barrier properties of the outer membrane, increasing resistance to bacteria. However, ZnO nanoplates act effectively against both gram-negative and gram-positive bacteria, inhibiting their growth [23]. Three mechanisms have been proposed for the antibacterial activity of ZnO: (1) the release of antibacterial ions from nanoparticles, (2) the interaction between nanoparticles and microorganisms, and (3) the generation of ROS by light irradiation [24]. However, the first mechanism of release of antibacterial ions such as Zn^{2+} , Mn^{3+} , and Fe^{3+} and the second mechanism of electrostatic interaction between microorganisms and NP have been also reported as antibacterial mechanisms in other nanoparticles [25,26]. However, ROS generation in ZnO is treated as a special major mechanism of antibacterial activity. ROS are generated according to a stepwise mechanism such as bacterial surface adsorption, electron/hole pair generation, oxygen/water reaction with the generated pair, and formation of various intermediates [27]. In addition, direct (ROS generation inside bacterial cells) and indirect (ROS generation outside bacterial cells) methods for ROS generation have been reported [28].

In a previous study, we confirmed that various reactive oxygen species, ROS ($\cdot\text{OH}$, $\cdot\text{O}^{2-}$, and H_2O_2), were generated from ZnO nanoplates, and it was revealed that $\cdot\text{O}^{2-}$ radicals were more important to antibacterial performance than $\cdot\text{OH}$ radicals [29]. Moreover, it was also found that oxygen vacancies have an important effect on ROS generation. However, it has not been clarified whether oxygen vacancies in ZnO nanoplates always have a positive effect on antibacterial performance, and what degree of oxygen vacancies is the most ideal. It is very difficult to reveal this, but there is no doubt that it is very interesting and meaningful. Therefore, this study attempted to synthesize ZnO nanoplates whose particle shapes were constantly controlled by fixing the capping agent NaOH at 4.0 M and changing

the crystallization temperature to 50, 100, 150, and 200 °C. Moreover, we wanted to find out what effect the minute oxygen vacancies in these particles have on the antibacterial activity against gram-negative and gram-positive bacteria. In addition, it was investigated how the light-induced charge separation and photoluminescence characteristics change due to the oxygen vacancies introduced into the ZnO nanoplates, and how the antibacterial performance changes accordingly.

The main objective of this study is to investigate the potential of oxygen-defective ZnO nanoplates as a photocatalyst for the enhanced degradation of MB and as an antibacterial agent. Specifically, the study seeks to evaluate the performance of this photocatalyst in comparison to conventional systems that are currently used for dye wastewater treatment and antibacterial applications in various industries. The study aims to provide a comprehensive understanding of the mechanisms underlying the enhanced photocatalytic and antibacterial activity of the oxygen-defective ZnO nanoplates, which will be achieved through a series of experiments and analyses. The results of these experiments will be used to evaluate the potential of the photocatalyst for real-world applications, such as in the treatment of industrial dye wastewater and in the development of novel antibacterial agents. The study will also aim to identify the optimal conditions for the synthesis of oxygen-defective ZnO nanoplates and to determine the factors that influence their performance, such as the size and morphology of the nanoplates, the concentration of the photocatalyst, and the intensity and wavelength of the light source used for the photocatalytic reaction. Overall, this study aims to contribute to the development of more efficient and sustainable systems for the treatment of dye wastewater and the prevention of bacterial infections by investigating the potential of oxygen-defective ZnO nanoplates as a promising alternative to current conventional systems.

2. Results and Discussion

2.1. Physical and Chemical Properties of ZnO Nanoplates

An XRD pattern of ZnO prepared using a hydrothermal process is shown in Figure 1a. All crystals were found to have hexagonal wurtzite structures in the XRD pattern [30], and no other crystalline phases were observed. Furthermore, as crystallization temperature increased, the position of peaks did not change but their intensity did, especially that of the (101) plane [31]. In the TEM image of ZnO-50 synthesized at 50 °C in Figure 1b, a mixture of sheet and thin rod shapes was seen. In particular, it is seen that the rod particles are aggregated like leaves toward the center. Perhaps, after the sheet particles are wound and turned into thin rod particles, these rod particles stick to each other sideways and grow into plate particles. All of the ZnO crystals obtained at temperatures above 100 °C exhibited a plate shape. As the crystallization temperature increased, the particles agglomerated with each other and slightly increased in size. This phenomenon was also seen in the SEM image of Figure 1c, and larger and distinct rhombic plates are aggregated in the ZnO-200 nanoplates than in the ZnO-100 nanoplates.

Furthermore, the ZnO nanoplate structures were confirmed by the HR-TEM analysis presented in Figure 2a. A SAED pattern and corresponding identified lattice fringes associated with ZnO from Figure 2b–d appeared with the interplanar distances of 0.260, 0.162, and 0.141 nm, which are ascribed to the (001), (110), and (200) plane of the ZnO phases. ZnO found a pure type of nanoplates clearly represented by elemental mapping. Figure 2e shows the elements used in the synthesis of ZnO, which are zinc and oxygen.

In Figure 3a, high-resolution XPS Zn2p and O1s spectra from ZnO-50 and ZnO-200 nanoplates are compared. The Zn2p spectrum in the ZnO particle was separated into two peaks attributed to Zn2p_{1/2} and Zn2p_{3/2}, located at about 1044 and 1021 eV [32]. In the ZnO-200 nanoplates, the Zn 2p peak was slightly shifted toward higher binding energies. This means that the ZnO-200 nanoplates are coordinated with more oxygen than the ZnO-50 nanoplates, indicating that more oxygen vacancies exist in the ZnO-50 nanoplates [33]. Perhaps because of the low crystallization temperature, the wurtzite structure of ZnO was not completely formed in the ZnO-50 nanoplates, resulting in large

O deficiency. Observations indicate that the O 1s spectra are asymmetric; therefore, each spectrum comprises three peaks located at 529.93 eV, 531.32 eV, and 531.9 eV [34,35]. It was observed from the XPS spectra that O_L peak at 529.93 eV corresponds to O^{2-} ions on the wurtzite structure of the Zn^{2+} ion array, which are bonded with either Zn atoms [36]. The O_V peak centered at 531.32 eV is usually assigned to O^{2-} in oxygen-deficient sites within the ZnO matrix, which is indicative of the corresponding oxygen vacancy concentration [37]. The O_C peak located at 531.9 eV is ascribed to some chemisorbed oxygen components in the form of H_2O , $-CO_3$, and OH^- groups [38]. As a result, the oxygen defect was found to be much greater in ZnO-50, while in ZnO-200, the oxygen defect decreased and the area of O_C increased. This confirms that the oxygen defect decreased in ZnO-200 compared to ZnO-50. The peaks in the ZnO-50 nanoplates shifted slightly toward lower energy, and the peak area of Zn-OH in particular increased slightly. This is related to OH groups adsorbed on the surface of ZnO-50 nanoplates, or to O vacancies in the Zn-O lattice [39]. As a result of quantitative analysis, it was confirmed that the Zn:O abundance ratio in ZnO-50 nanoplates was 52.08(1):47.92 (0.92), indicating about 8% of O vacancies, and the Zn:O abundance ratio in ZnO-200 nanoplates was 51.76(1):48.24 (0.93), indicating the presence of about 7% of O vacancies.

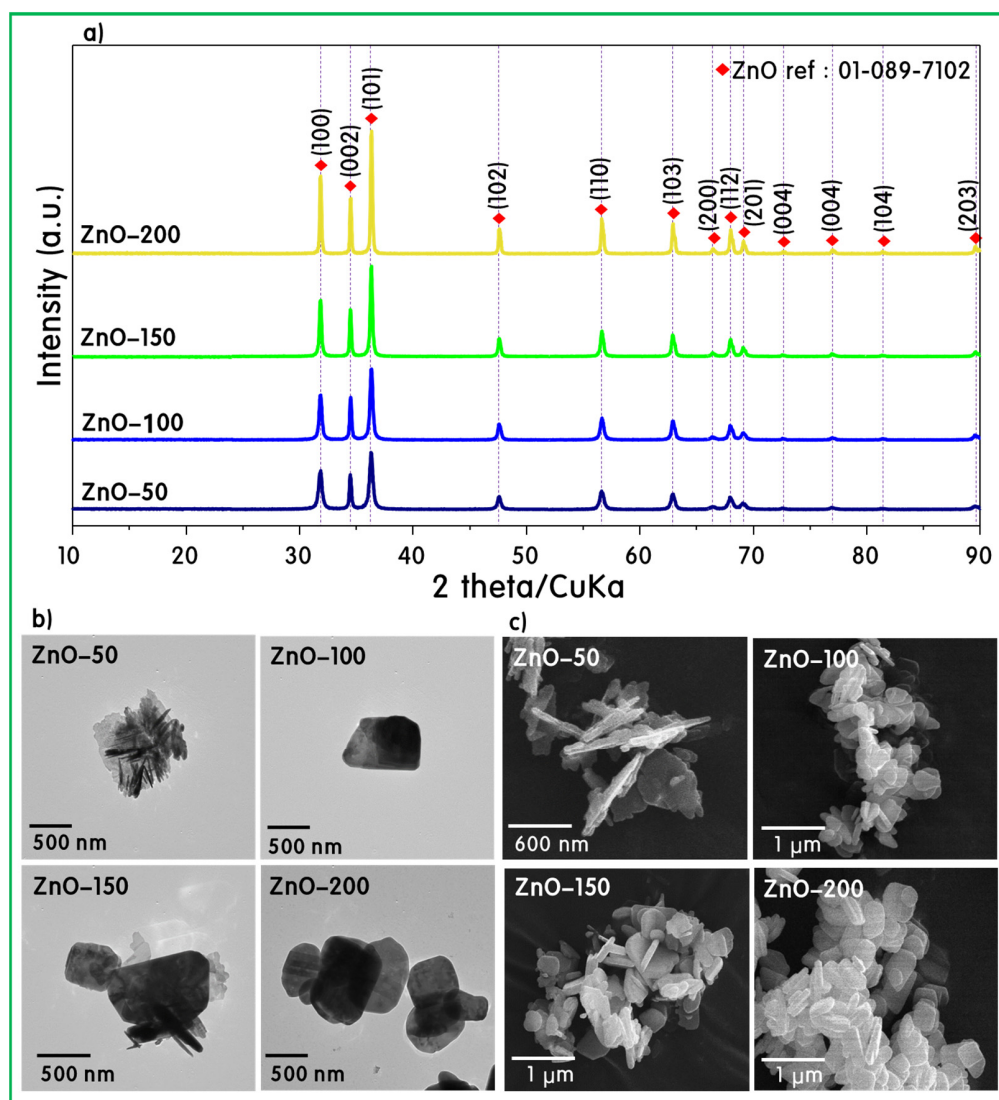


Figure 1. (a) XRD patterns, (b) TEM images, and (c) SEM images for the ZnO nanoplates.

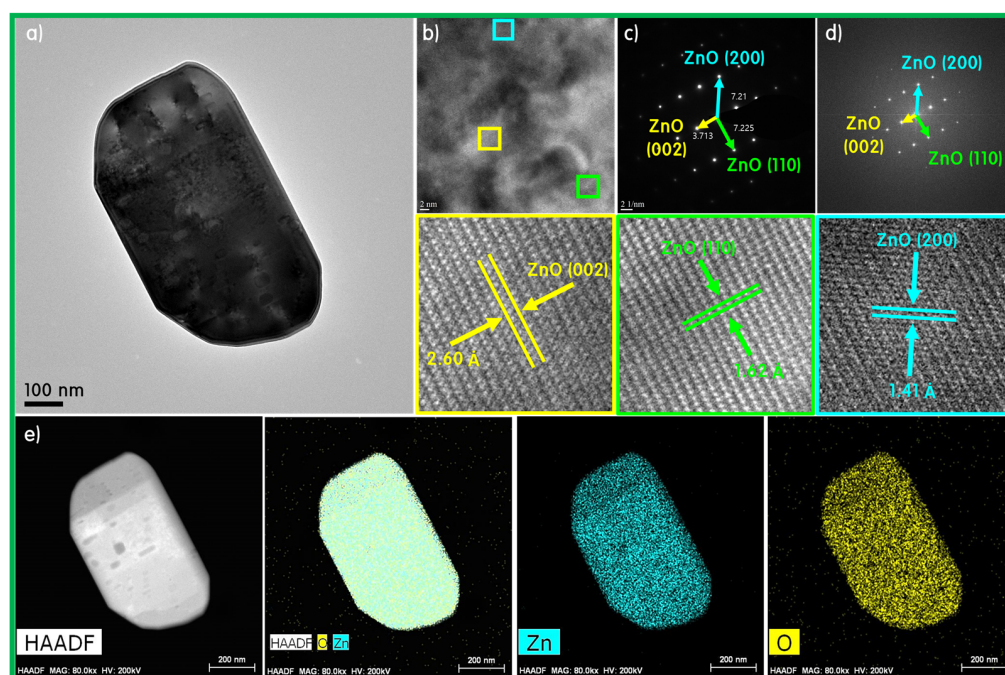


Figure 2. (a) HRTEM image of the obtained ZnO nanoplates; (b–d) SAED pattern and corresponding identified lattice fringes associated with ZnO; (e) HAADF image as well as elemental mapping of the ZnO nanoplates.

Figure 3b shows the FT-IR spectrum of the ZnO nanoplates. The bands located at 450 and 560 cm^{-1} seen in all samples correspond to the Zn-O-stretching vibration mode in the ZnO crystal [40] and became sharper as the crystallization temperature increased. On the other hand, the OH-bending and stretching vibration modes appeared cleanly at 1620 and 3420 cm^{-1} [41]. As the crystallization temperature increased, the OH-bending mode had no tendency, but the OH-stretching mode became smaller. This means that a lot of $\text{Zn}(\text{OH})_2$ or $\text{Zn}(\text{OH})_4^{2-}$ phases on the crystal surface disappeared, indicating that as the crystallization temperature increased, the number of ZnO surface defects decreased. Figure 3c shows the Raman spectra of ZnO-50 and ZnO-200 nanoplates. Raman spectroscopy is a powerful tool for analyzing structural defects. The space group P6₃mc of wurtzite ZnO has six Raman-active optical phonon modes in the center of the Brillouin zone with $\Gamma_{\text{opt}} = 2A_1 + 2E_1 + 2E_2$ [42]. Here, E_1 , E_2 and A_1 are the first-order Raman active modes, the E_1 and A_1 polar modes are divided into longitudinal optical (LO) and transverse optical (TO) components, and finally there are low and high E_2 non-polar modes. For both ZnO, peaks appeared in the low-frequency region of 334, 386, and 443 cm^{-1} [43]. The peak at 334 cm^{-1} is related to multiphoton processes and is assigned $E_2(\text{high})$ - $E_2(\text{Low})$ arising from zone boundary phonons. The peaks at 386 and 542 cm^{-1} correspond to the $A_1(\text{TO})$ mode and are caused by some defects such as Zn interstitials and oxygen vacancies. A sharp and noteworthy peak at 443 cm^{-1} is assigned to the high mode of E_2 . This is the strongest mode in the wurtzite crystal structure and is due to the motion of oxygen atoms in the ZnO lattice. Peaks at 588, 676, and 690 cm^{-1} are characteristic of the $E_1(\text{LO})$ mode caused by lattice defects or metal doping, and are also interpreted as signs of oxygen deficiency [44]. From these results, it was confirmed that large Zn interstitial defects and small oxygen vacancies exist in the Zn-200 nanoplates, whereas large Zn interstitial defects and large oxygen vacancies exist in the Zn-50 nanoplates.

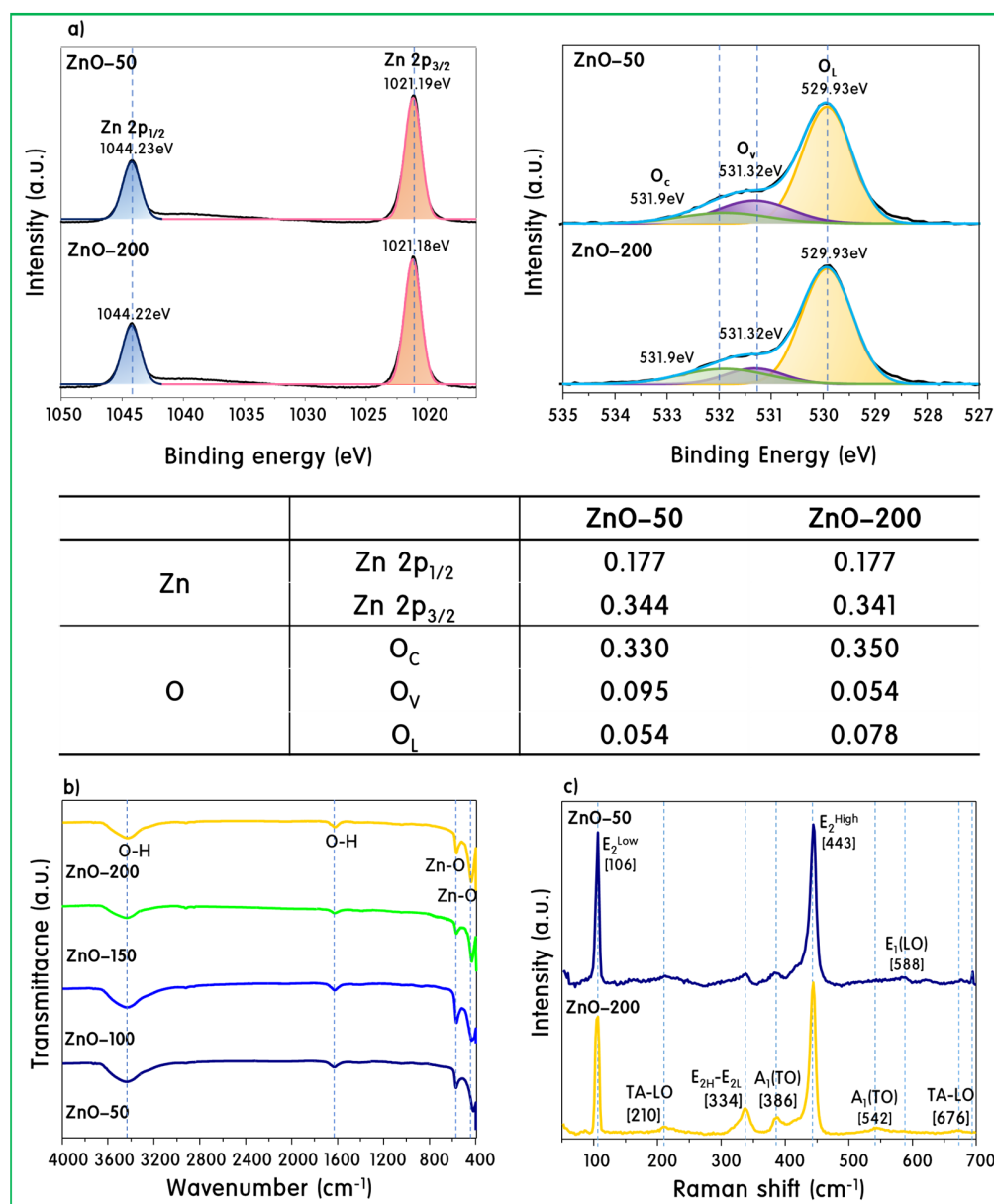


Figure 3. (a) XPS spectra, (b) FTIR spectra, (c) Raman spectra of ZnO nanoplates.

2.2. Spectroscopic Characteristics of Synthesized ZnO Nanoplates

In Figure 4a, the DRS-UV-visible absorbance peaks of the ZnO nanoplates show a maximum at about 380 nm. The optical bandgap energy (E_g) of ZnO was estimated from the Tauc plot [45], as shown in Figure 4b. The calculated band gaps of the ZnO-50, ZnO-100, ZnO-150, and ZnO-200 nanoplates were 3.23, 3.22, 3.21, and 3.19 eV, respectively, and the band gap narrowed as the crystallization temperature increased. In the XPS-VB curve of Figure 4c, the VBM (VB maximum) of the ZnO-50 and ZnO-200 nanoplates was 2.65 and 2.50 eV, respectively. Subtracting the band gap obtained in Figure 4b from these values, the location of the CB minimum (CBM) is determined to be -1.58 and -0.69 eV for ZnO-50 and ZnO-200 particles, respectively. From these results, the band potential energy positions of ZnO-50 and ZnO-200 nanoplates can be drawn as shown in Figure 4d. Although the band gap decreased slightly in the ZnO-200 nanoplates compared to the ZnO-50 nanoplates, it could be negligible. However, the VBM energy level shifted further downward by the oxygen deficiency (V_o) in ZnO-50 nanoplates [46]. At the same time, the CBM energy position also moved downward. However, since the energy bands in the

two oxygen-deficient ZnO nanoplates contained oxidation-reduction potentials for water decomposition, it seems reasonable to generate ROS in the photoactive reaction.

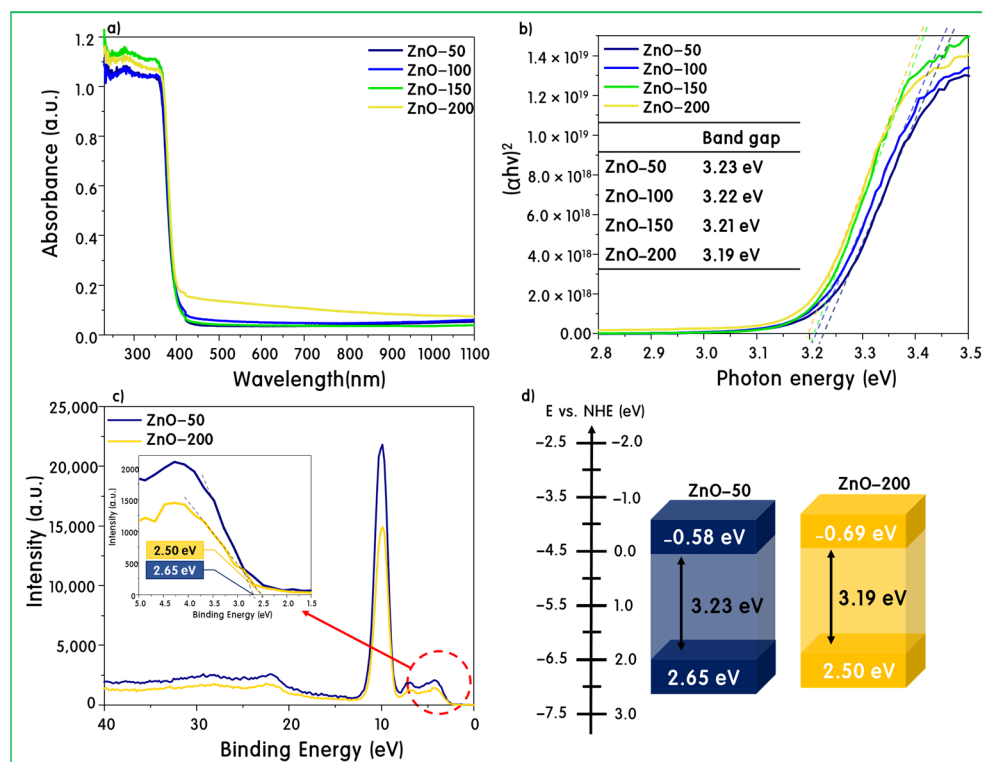


Figure 4. (a) DRS-UV-Visible absorption spectra, (b) Tauc's plot curves, (c) XPS valence band, and (d) energy diagrams for the bandgap positions of the samples.

In the PL spectrum of Figure 5a, the ZnO nanoplates exhibited large near UV emission and broad long-wavelength emission bands. In general, the intensity, location, and proportion of PL imply the presence of major defects such as zinc vacancies, single and double ionized oxygen vacancies, and lattice oxygen vacancies [47]. It has recently been shown that changes in oxygen sites in ZnO nanoplates lead to changes in antibacterial properties [48]. In this work, peaks appearing at 450 and 470 nm are related to oxygen vacancies or defects of interstitial zinc ions, and emission at 490 nm is due to the transition between photoexcited holes and single ionized oxygen vacancies [49]. As the crystallization temperature was lowered, the intensity of the overall peaks increased, and the oxygen vacancies became larger. On the other hand, to describe the exciton lifetime in ZnO nanoplates, time-resolved photoluminescence (TRPL) is measured as shown in Figure 5b. The difference in decay constant value between the two particles was 2527 ns, indicating a longer decay rate in the ZnO-200 nanoplates. This means that fast recombination of photo-generated carriers occurs in ZnO-50 nanoplates with fast decay constants, and slow recombination of photo-generated carriers in ZnO-200 nanoplates with slow decay. The slow recombination may be due to oxygen vacancy acting as an electron trapping center for carrier relaxation. However, excessive oxygen vacancy seems to have the opposite effect [50]. Meanwhile, in Figure 5c, the intensity of the photocurrent density increased in the order of ZnO-50 < ZnO-100 = ZnO-150 < ZnO-200 nanoplates. When irradiated with visible light, the photocurrent density is the highest and most stable in ZnO-200 nanoplates, which means that ZnO-200 nanoplates have a better visible light response and more efficient charge separation than other particles. Moreover, the time-dependent decay rate of the photocurrent density, shown in Figure 5d, remained stable for all particles up to 330 s. The ZnO-200 nanoplates, in particular, increased slightly with time. In the end, it was found that excessive oxygen vacancies that were not compensated could become

stronger carrier recombination centers under light irradiation and hinder the separation of photocharges [51].

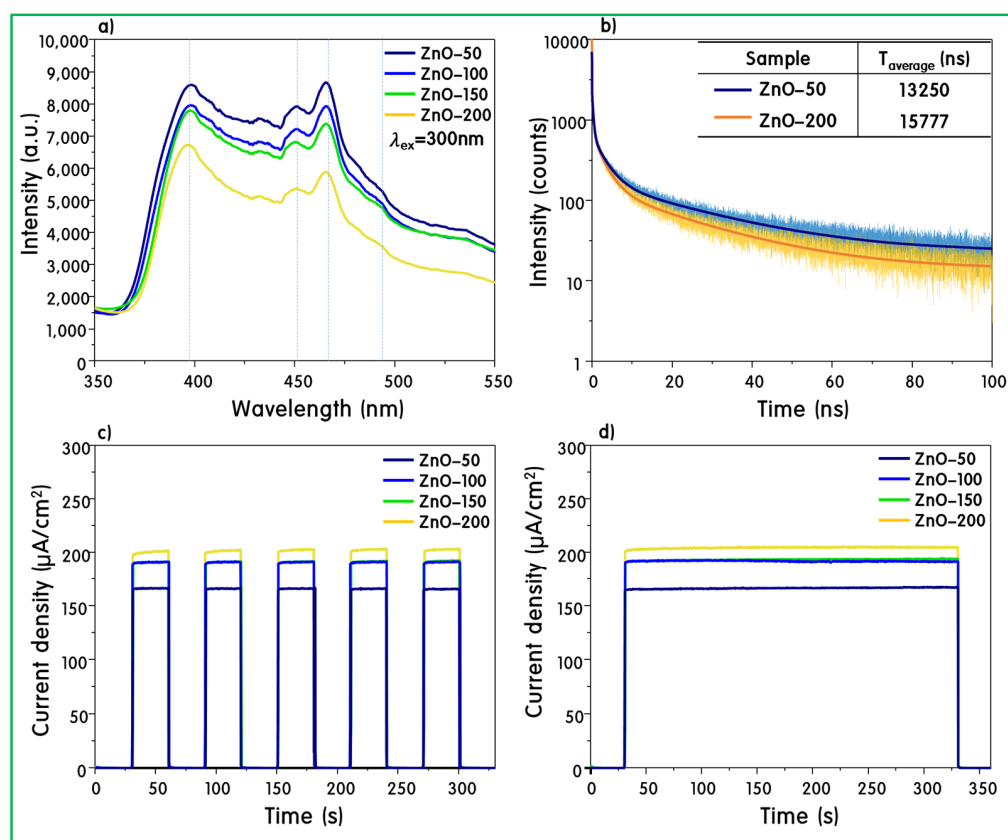


Figure 5. (a) PL curves. (b) Time-resolved PL curves for ZnO-50 and ZnO-200 nanoplates. (c) Photocurrent density cycle curves. (d) Time-resolved photocurrent density curves of synthesized ZnO nanomaterials.

In order to exhibit photoactivity, ROS release during photoreaction is required, and in particular, the generation of $\cdot OH$ and $\cdot O_2^-$ radicals induced by water adsorption is very important. Therefore, as shown in Figure 6, the water desorption according to the temperature change in the obtained ZnO nanoplates was observed through the TGA curve. At this time, the activation energy for water desorption was calculated using the Ozawa method [52]. In Figure 6a, as the crystallization temperature increased, the TGA curves on ZnO nanoplates became more widely distributed forward high temperatures. In Figure 6b, high water desorption activation energy means good water adsorption capacity on the ZnO crystal surface. The water desorption activation energy in ZnO-200 nanoplates was about 15 kJ/mol higher than that in ZnO-50 nanoplates. Therefore, it was predicted that more water would be adsorbed on the surface of ZnO-200 nanoplates, and more ROS would be generated during the photocatalytic reaction.

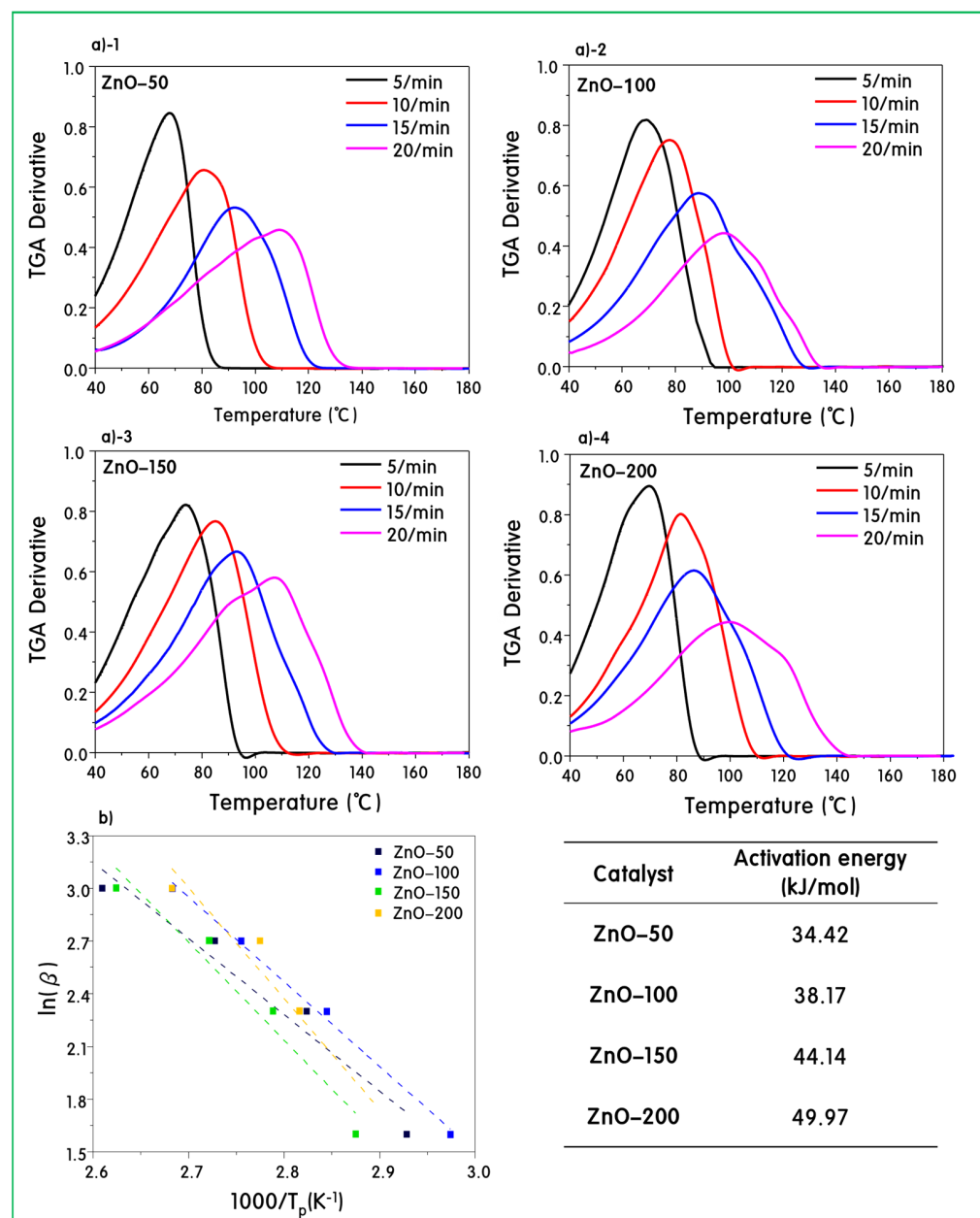


Figure 6. Water activation energy (a)–(d) Change in water desorption graph according to heating rate for each catalyst for TGA curve (b) Relationship between temperature and rate constant for water desorption for each catalysts.

2.3. Photocatalytic and Bactericidal Activity in Synthesized ZnO Nanoplates

In order to compare the photocatalytic activity of ZnO nanoplates, methyl blue (MB) decomposition experiments were performed per hour for each particle as shown in Figure 7a-1. The initial concentration of MB was fixed at 100 mg/L, and the decomposition of MB over time was confirmed as a peak decrease in the absorption region of 600–700 nm. In the ZnO-50 nanoplates, the absorption peak of MB was still large even after 5 h, indicating that the decomposition was still less. However, in ZnO-100 nanoplates, the absorption peak of MB rapidly decreased with time. Moreover, in the case of ZnO-200 nanoplates, the rate of elimination was faster, and the MB absorption peak almost disappeared after 5 h, and MB color was hardly seen. The change in MB removal concentration per hour for the 5 h reaction is shown in Figure 7a-2. The results indicate that synthesized ZnO nanoparticles demonstrated good photocatalytic activity against MB dye under visible light and the results are compared with earlier reports (Table 1). When ZnO nanoplates have too

many oxygen vacancies, the shallow trapping effect that enhances photocatalytic activity is diminished, and the deeper trapping states become more prominent. This can result in increased recombination of photo-generated electron-hole pairs, leading to reduced photocatalytic activity. Furthermore, too many oxygen vacancies can create more mid-gap states, which can trap both electrons and holes and can increase the rate of charge recombination. This results in decreased photocatalytic activity and poor efficiency. In addition, excessive oxygen vacancies can also lead to a reduction in the surface area available for photocatalytic reactions. This is because the formation of oxygen vacancies can cause the nanoplates to aggregate, leading to a decrease in surface area and a reduction in the number of active sites available for catalytic reactions.

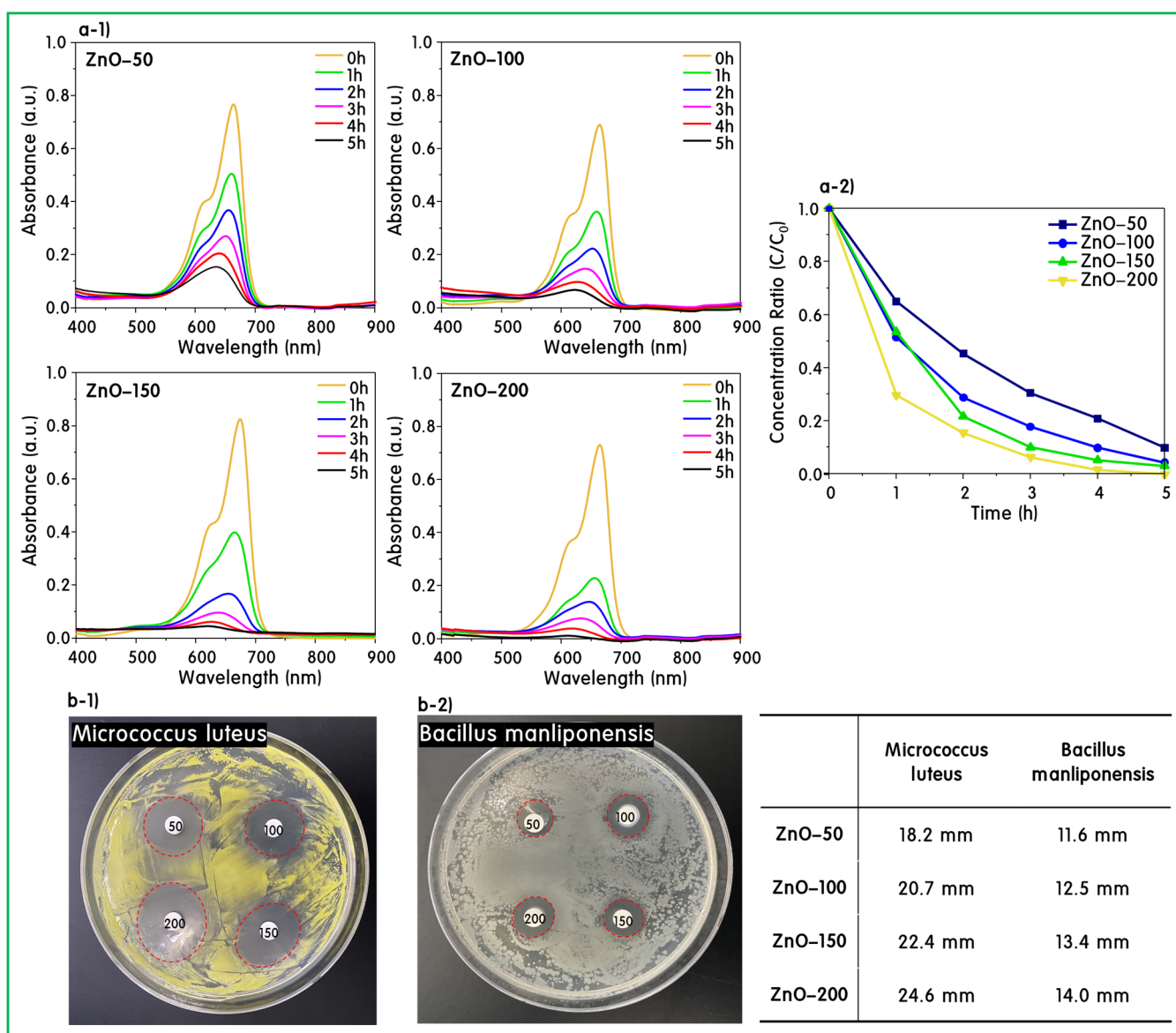


Figure 7. (a-1,a-2) MB dye decomposition performance (b-1,b-2) Antibacterial performance according to the ZnO nanoplates each temperature synthesized.

In summary, while oxygen vacancies can be beneficial for photocatalytic activity, excessive amounts of oxygen vacancies can have negative impacts on the photocatalytic activity. The optimal amount of oxygen vacancies may depend on the specific morphology and size of the ZnO nanoplates, as well as other factors such as the reaction conditions and the nature of the reactants [53,54]. Therefore, it is important to carefully control the oxygen

vacancies and to optimize the conditions for photocatalytic reactions to achieve the best possible activity.

It can be seen that MB is decomposed more quickly in ZnO-200 nanoplates with fewer oxygen vacancies. MB is ionized into Na^+ and $\text{C}_{14}\text{H}_{14}\text{N}_3\text{O}_3\text{S}$ anions in aqueous solution. In the end, during the photoreaction, when Na^+ is adsorbed on the CB of ZnO as an electron scavenger, more activated $\cdot\text{OH}$ radicals in ZnO-VB are generated to decompose the $\text{C}_{14}\text{H}_{14}\text{N}_3\text{O}_3\text{S}$ anion. The ZnO photocatalyst produces hydroxyl radicals ($\cdot\text{OH}$) when the water molecules are exposed to UV light and ZnO photocatalyst. In a process known as oxidation, these hydroxyl radicals react with MB to lead to its decomposition. The reaction involves the transfer of an electron from MB dye to the hydroxyl radical, forming a MB radical cation and a hydroxide ion. Further oxidation of the methylene blue radical cation occurs when it reacts with another hydroxyl radical, decomposing it into smaller, less colored molecules. Similar mechanisms are reported by other researchers [55,56]. Overall, the reaction can be represented as:

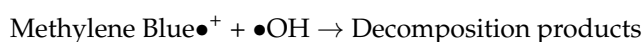
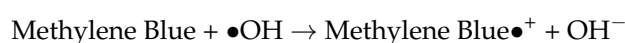


Table 1. Comparative study of photocatalytic degradation of MB under visible light.

Catalyst	Condition	Light Source	Degradation	Ref.
ZnO	MB 100 ppm	100 mW/cm ² xenon lamp (One-sun region)	5 h 95%	This study
ZnO	MB 1.25 ppm	UV lamp	7 h 100%	[57]
Ag-ZnO	MB 20 ppm	100 W Xenon lamp	4 h 96%	[58]
Fe-ZnO	MB 20 ppm	150 W mercury light	3 h 92%	[59]
Cd-ZnO	MB 6 ppm	Visible light irradiation	2 h 95%	[60]
N-doped ZnO	MB 10 ppm	AM 1.5 simulated solar light	2 h 99%	[61]

Meanwhile, the antibacterial activity of ZnO nanoplates against the pathogen *Micrococcus luteus* and the marine bacterium *Bacillus maniponensis* was measured. Figure 7b shows the inhibition diameter, which means antibacterial performance, and summarized in the table. Antibacterial array disks show zones of inhibition for all ZnO samples. The inhibition diameters for the *Micrococcus luteus* pathogen were 18.2, 20.7, 22.4, and 24.6 mm for the ZnO-50, ZnO-100, ZnO-150, and ZnO-200 nanoplates, respectively. The inhibition diameters for the marine bacterium *Bacillus maniponensis* were 11.6, 12.5, 13.4, and 14.0 mm for the ZnO-50, ZnO-100, ZnO-150, and ZnO-200 nanoplates, respectively. This result suggests that ZnO nanoplates exhibit stronger bactericidal activity against pathogens than marine bacteria. Despite the fact that photocatalysts have a lower antibacterial activity than standard drugs, their unique properties, including selectivity, stability, versatility, and environmental friendliness, make them an attractive choice for antibacterial applications across many fields.

The antibacterial and sterilizing effects were observed per hour according to the presence or absence of light (400 nm LED) for *Staphylococcus aureus*, a gram-positive bacterium, and *Escherichia coli*, a gram-negative bacterium, for ZnO-200 nanoplates showing the best photocatalytic performance. The results are presented in Figure 8a,b. First, for the control group without a catalyst, it was confirmed that the bacteria tended to continually grow both when light was irradiated and when light was not irradiated. In particular, it can be seen that the bacteria are partially sterilized by the light, since the bacteria grow less in the presence of light than when there is no light. In addition, the fertility of gram-negative bacteria, *Escherichia coli*, was stronger than that of gram-positive bacteria, *Staphylococcus aureus*.

On the other hand, in the presence of ZnO-200 nanoplates, 86.67% of sterilization effect was shown after 3 h when light was not irradiated on *Staphylococcus aureus*, a gram-positive bacteria. On the other hand, when irradiated with light, a high bactericidal activity of 96.95% was shown. Regarding *Escherichia coli*, which is a gram-negative bacterium, 74.66% of sterilization effect was obtained after 3 h when light was not irradiated, and 95.83% of bactericidal activity was obtained after 3 h when light was irradiated. This result means that gram-positive bacteria with thin cell walls are much easier to sterilize than gram-negative bacteria with thick cell walls, and that when irradiated with light, the bactericidal effect is higher than when light is not irradiated. As shown in Scheme 1, three mechanisms have been proposed for the antibacterial activity of ZnO: (1) the release of antibacterial ions from nanoparticles, (2) the interaction between nanoparticles and microorganisms, and (3) the generation of ROS by light irradiation [24]. The photocatalytic reaction and antibacterial mechanism on ZnO nanoplates have already been reported in a previous study [29]. What we want to emphasize in this study is that the generation of ROS was rather suppressed in ZnO-50 nanoplates with excessive defects, and eventually the antibacterial performance was reduced. Still, we propose that the antibacterial activity is related to ROS generation: when there is a defect in the particle crystal, the defect can act as an electron trapper to facilitate the flow of photoinduced charges and eventually generate more ROS, resulting strong antibacterial properties. However, too many oxygen vacancies cause instability of the crystal and there is a risk of decreasing activity over time, and too many vacancies can act as exciton recombination sites and hinder ROS generation [62].

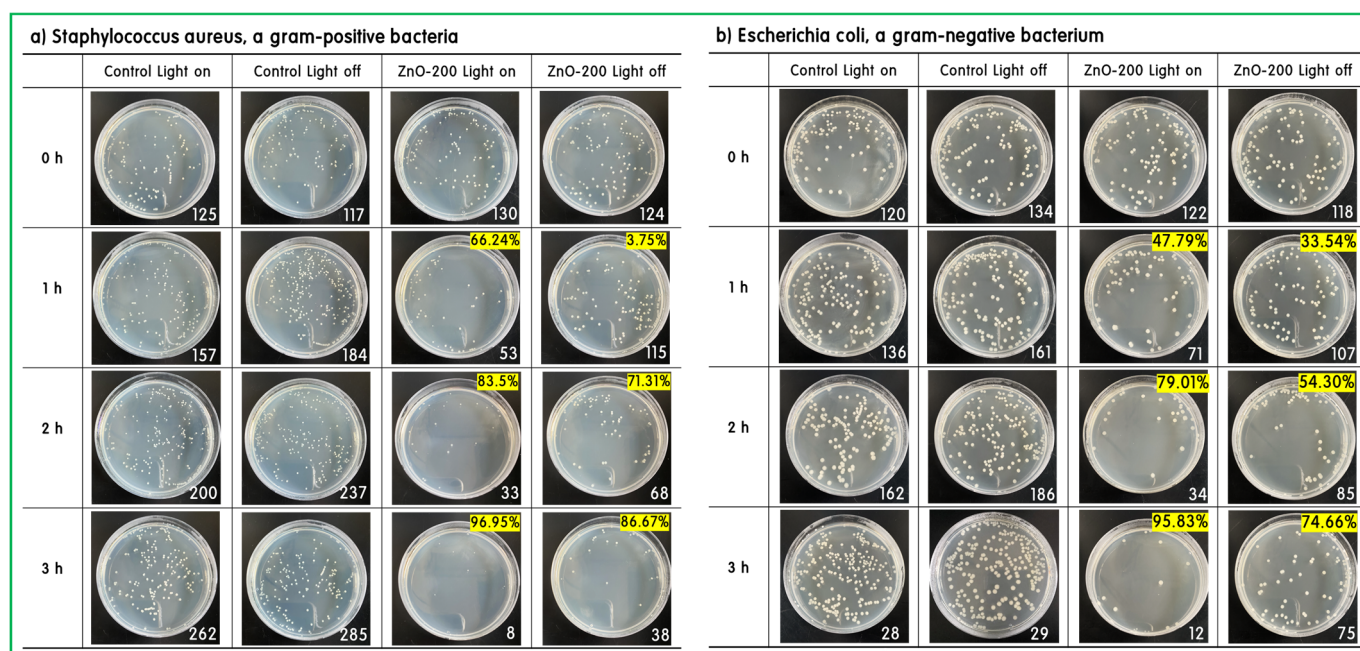
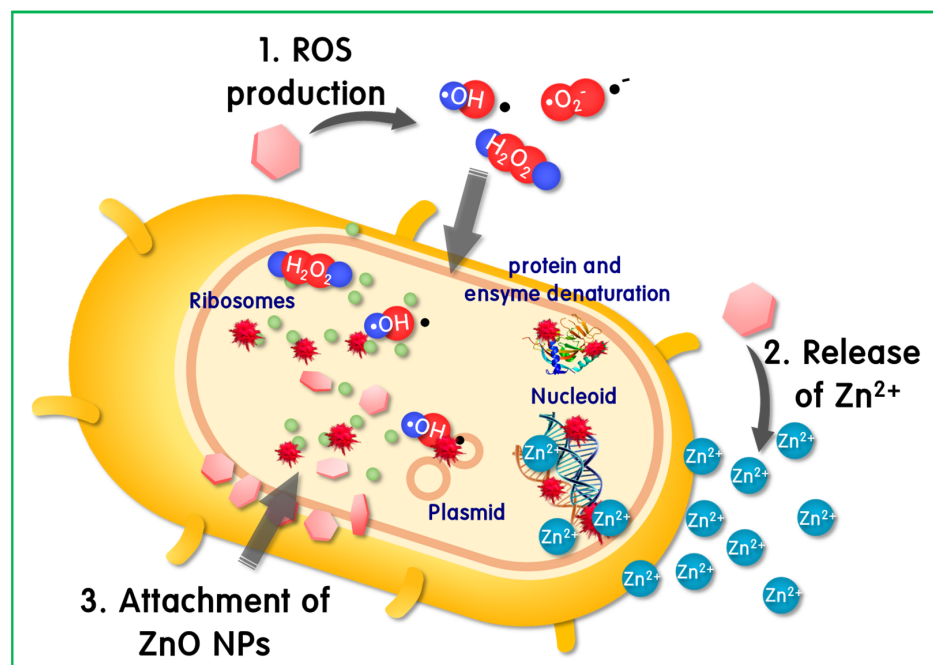


Figure 8. Sterilization capacity of ZnO-200 catalyst against (a) *Staphylococcus aureus*; and (b) *Escherichia coli*.

Electrochemical Impedance Spectroscopy (EIS) is a powerful tool for investigating charge carrier trapping, transport, and mobility in oxygen-deficient ZnO samples. The impedance spectrum obtained from EIS can provide information about the resistance and capacitance of the material related to charge transport characteristics. Analysis of the impedance spectrum can identify the electrical properties related to charge carrier trapping, such as the capacitance and resistance of charge trapping sites. In addition, impedance spectrum analysis can reveal the mobility of charge carriers related to the charge transport properties of the material.

To evaluate the charge transport dynamics of the sample, we performed an electrochemical impedance spectroscopy (EIS) test using a typical three-electrode system with a

counter electrode Pt and a reference electrode RHE. The obtained Nyquist plot was fitted to Randle's equivalent circuit model, and the drawn semicircles represent the charge transfer resistance.



Scheme 1. Proposed antibacterial activity mechanism in ZnO nanoplates.

The smaller the radius of the Nyquist circle, the lower the charge transfer resistance, and the size of the semicircle was smaller in the order of ZnO-50, ZnO-100, ZnO-150, and ZnO-200 (Figure 9). As a result, the ZnO-200 sample showed the lowest resistance, promoting the movement of surface electrons and facilitating the separation of photo-generated electron-hole pairs, thereby enhancing photocatalytic activity. In other words, an appropriate (not excessive) amount of oxygen vacancy on the surface acts as a charge transport channel, improving the separation of electrons.

To estimate the charge transfer pathways on the surface of ZnO-50 and ZnO-200 nanoplates, spin-trapping ESR analysis using DMPO as a free radical trapping agent was performed. $\cdot\text{OH}$ and $\cdot\text{O}_2^-$ radicals were detected over time (0, 10, 20, and 40 min), and the results are shown in Figure 10a,b. In Figure 10a-1, a small signal corresponding to the DMPO- $\cdot\text{OH}$ radical was observed as soon as the light was irradiated. The signal increased significantly with increasing irradiation time. In the ZnO-200 nanoplates in Figure 10b-1, the signal corresponding to the DMPO- $\cdot\text{OH}$ radical increased more significantly than in the ZnO-50 nanoplates. This means that more OH radicals are generated on the surface of ZnO-200 nanoplates than on the surface of ZnO-50 nanoplates. That is, when electrons present in VB of ZnO-200 nanoplates are excited to CB in the presence of light, $\cdot\text{OH}$ radicals are generated in VB-holes, and the amount of radicals generated increases over time. In Figure 10a-2, the DMPO- $\cdot\text{O}_2^-$ signal from ZnO-50 nanoplates was observed more clearly than that from DMPO- $\cdot\text{OH}$. Moreover, the signal intensity increased more significantly with the irradiation time. Moreover, this tendency was more evident in the ZnO-200 nanoplates in Figure 10b-2. This means that more electrons excited from VB of ZnO-200 nanoplates were collected to CB under light irradiation. The fact that the DMPO- $\cdot\text{O}_2^-$ signal after 40 min in both particles was stronger than that of DMPO- $\cdot\text{OH}$ signal indicates that $\cdot\text{O}_2^-$ radicals are generated more effectively than $\cdot\text{OH}$ radicals on the surface of ZnO. From these results, we determined that more ROS were generated on ZnO-200 nanoplates with appropriate defects than on the surface of ZnO-50 nanoplates with excessive defects, which influenced the photocatalytic activity and antibacterial performance.

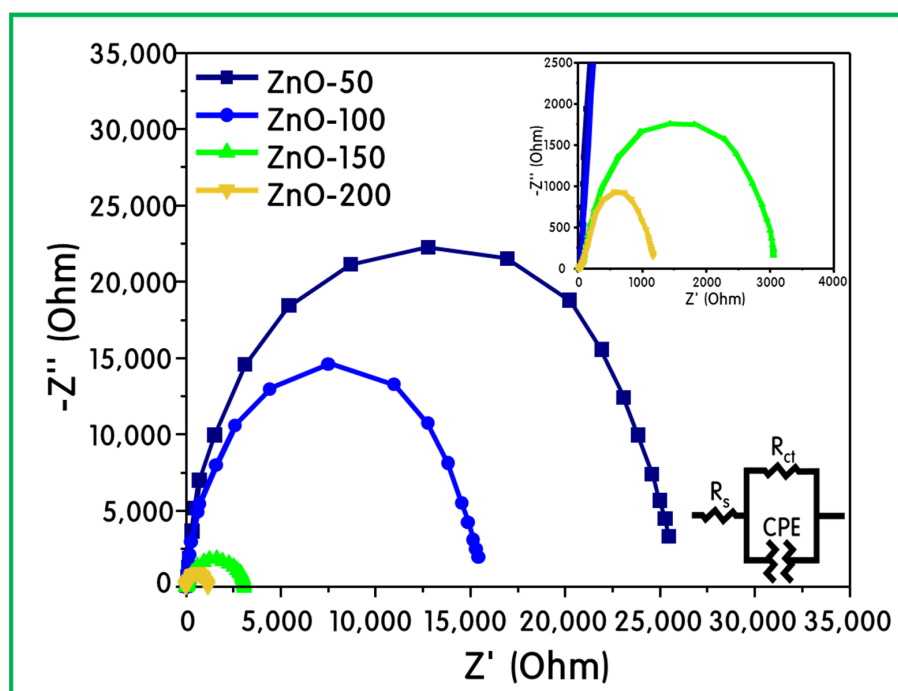


Figure 9. Electrochemical impedance spectra of ZnO-50, ZnO-100, ZnO-150, and ZnO-200 samples and its equivalent circuit.

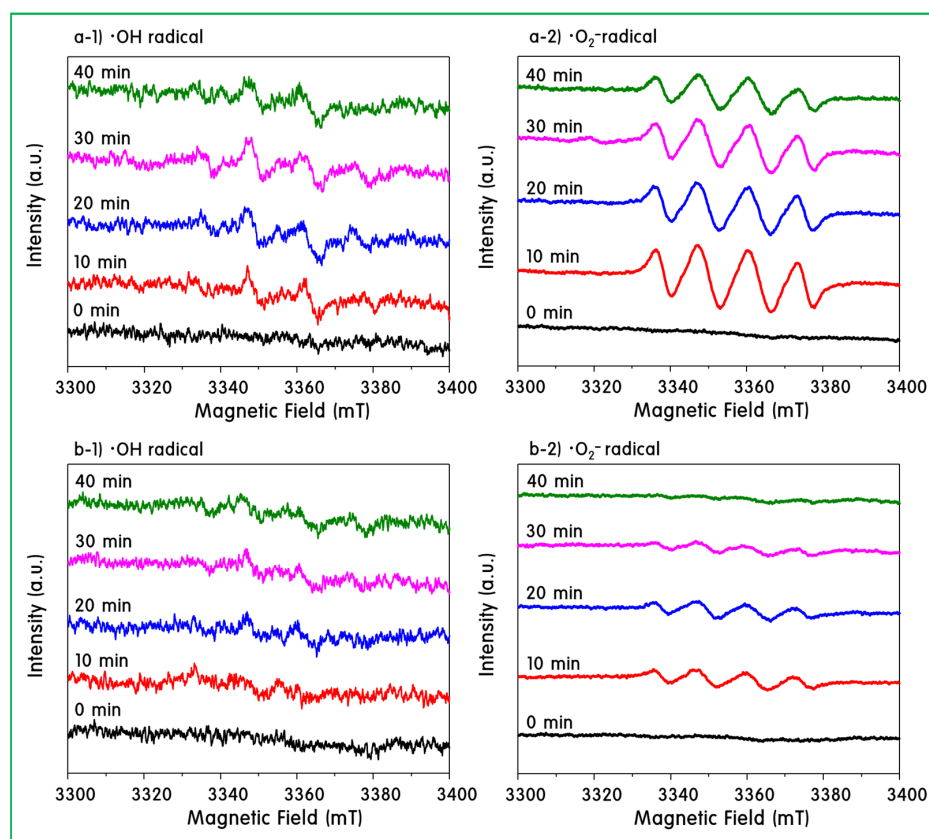


Figure 10. Spin-trapping ESR spectra in the presence of spin-trapping agent DMPO (**a-1,a-2**) ZnO-200 nanoplates DMPO-radical signals according to time change, (**b-1,b-2**) ZnO-50 nanoplates DMPO-radical signals according to time change.

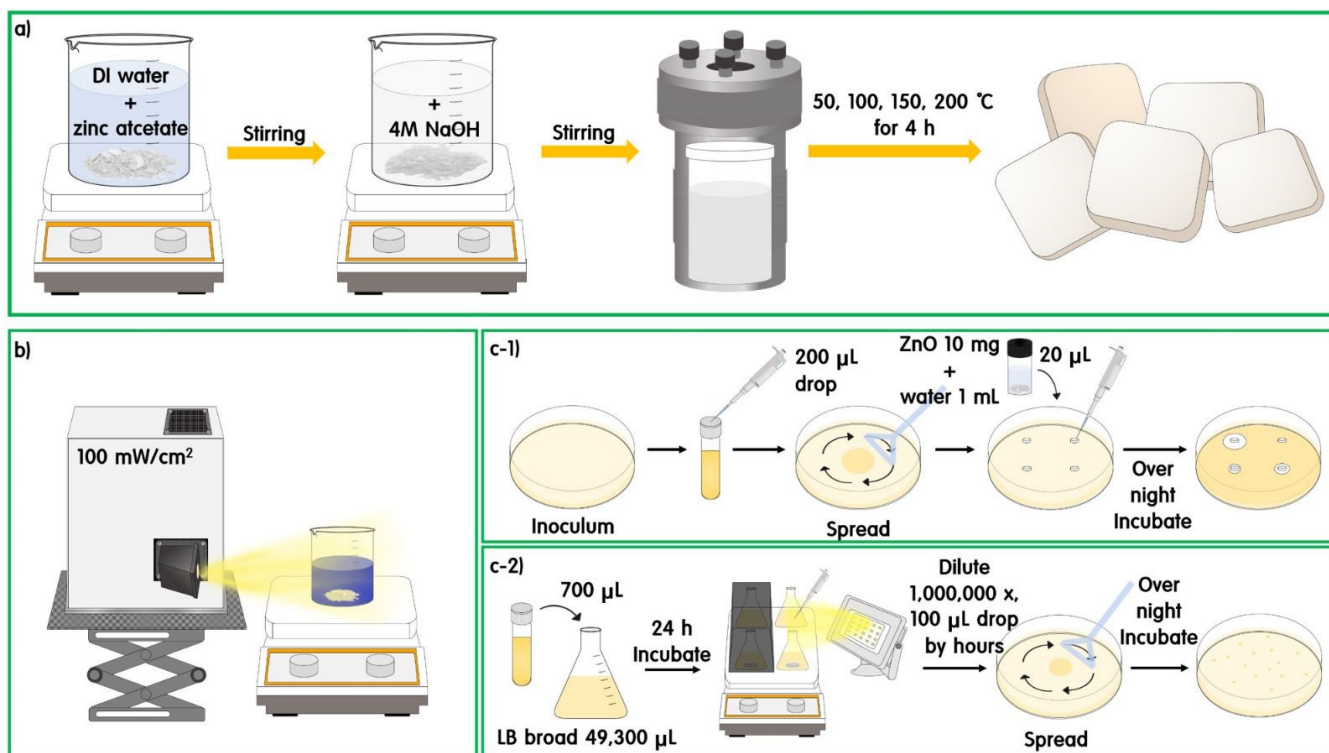
3. Materials and Method

3.1. Materials

All chemicals used were of analytical grade. Zinc acetates ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, 99.9%, 5,5-Dimethyl-1-pyrroline N-oxide ($\geq 97\%$) were purchased from Sigma Aldrich (Milan, Italy). Sodium hydroxide (98%) was purchased from Junsei Co., Tokyo, Japan). Water of reagent grade was produced with a Millipore purification pack system (MilliQ water, Sigma Aldrich, Milan, Italy).

3.2. Synthesis of ZnO Nanoparticle

The synthesized ZnO nanoplates were synthesized by the hydrothermal method as shown in Scheme 2a. A total of 1.0 M of zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, 99.9%, Sigma Aldrich, Milan, Italy) was dissolved in deionized water and stirred at room temperature for 1 h. To this, 4.0 M NaOH (98%, Junsei Co., Tokyo, Japan) was added and stirred for 1 h until uniform. After transferring these solutions to an autoclave, they were put into an electric furnace set at 50, 100, 150, and 200 °C, respectively, and heat-treated for 4 h. Finally, after cooling the electric furnace to room temperature, the autoclave lid was opened to filter the powder particles. These filtered particles were washed three times with deionized water to obtain four types of ZnO powders: ZnO-50, ZnO-100, ZnO-150, and ZnO-200. These particle powders were used as catalysts after drying for 12 h in a drying oven set at 50 °C.



Scheme 2. (a) Synthesis process of ZnO nanoplates by hydrothermal method, (b) photolysis system for MB, (c-1,c-2) schematic diagram of the antibacterial test process.

The mechanism of oxygen vacancy formation in ZnO during thermal synthesis in high-pH environments is a complex process that is not yet fully understood. However, some studies suggest that the formation of oxygen vacancies in ZnO during the synthesis process is closely related to the pH and temperature of the reaction solution. ZnO is an ionically bonded crystal that can form oxygen deficiency structures called “oxygen vacancies”. Oxygen vacancies are defect structures in which oxygen atoms are missing

from the ZnO structure, and they have a significant impact on the material properties and reactions.

In high-pH environments during hydrothermal synthesis, the concentration of hydroxide ions in the reaction solution is high, which can react with ZnO to form zinc hydroxide and promote the crystallization of ZnO. Additionally, at high temperatures, the crystallinity of ZnO increases, while at low temperatures, the crystallinity decreases, which can lead to the formation of oxygen vacancies at the grain boundaries. The mechanism of oxygen vacancy formation in ZnO is still under investigation, but studies on the influence of high-temperature oxygen atom mobility and defect formation in the crystal structure can provide important theoretical knowledge for understanding the formation and properties of these defects.

A hydrothermal reaction, as we are aware, is a chemical reaction using water to create water in a reactivated pressure vessel, an operation that requires high temperatures and high pressures currently. Suctioned pressure vessels exert different pressures depending on their temperature: 0.12 atm for 50 °C, 1 atm for 100 °C, 4.13 atm for 150 °C, and 15 atm for 200 °C. With an increase in temperature, pressure increases in the closed vessel, and reaction rates increase with increase in temperature in the vessel. High-temperature ZnO has a much larger diameter than ZnO obtained at this degree of purity, which leads to enhanced nucleation and growth, leading to rapid growth both lateral and axial. Moreover, high pH environments usually contain many hydroxide ions, which can enhance crystallinity and enhance crystallization. Radicals may form, however, if a hydroxide ion starts to lag after arriving over a long time at high temperatures. ZnO crystallization can be improved with these radicals by removing atoms from crisis [63].

3.3. Characterizations

The crystal structure of the synthesized ZnO was confirmed using X-ray diffraction (XRD, Miniflex 600, Rigaku, Tokyo, Japan, Cu K α source), and the presence of organic molecules present on the ZnO surface was confirmed using Fourier Transform Infrared (FT-IR) spectroscopy (Nicolet iS10 spectrometer, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Absorbance was determined by diffuse reflectance spectroscopy-ultraviolet (DR)-UV-visible spectrophotometer (Neosys-2000, SCINCO, Daejeon, Korea) Korea. Photoluminescence (PL) spectra were taken using a PerkinElmer fluorescence spectrophotometer with a He-Cd laser source with an excitation wavelength of 320 nm. Time-resolved photoluminescence (TRPL, Carl Zeiss, Oberkochen, Germany) measurements were performed using a confocal microscope (MicroTime 200; PicoQuant GmbH, Berlin, Germany) with an excitation wavelength of 360 nm. Photocurrent density was confirmed using a Sun 2000 solar simulator (IviumStat, ABET Technologies, 400 nm LED).

In the photocatalytic process, the main reactive species (radicals and holes) were detected through radical scavenging experiments. To detect OH and $\cdot\text{O}^{2-}$ radicals, 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) was used as a free radical scavenger, and a spin capture ESR assay was performed.

3.4. Photocatalytic Activity and Antibacterial Assay

As shown in Scheme 2b, the photocatalytic activity of ZnO was evaluated in a photoreaction system using the decomposition of MB dye. Under a 100 mW/cm² xenon lamp at room temperature, 1 g/L of ZnO photocatalyst was added to an aqueous solution containing MB dye at a concentration of 100 mg/L. Before light irradiation, the solution containing the ZnO catalyst was left in the dark for 30 min to reach the dye adsorption-desorption equilibrium on the ZnO surface. The suspension was then exposed to a xenon lamp at a reactor-lamp distance of 5.0 cm to decompose the dye. During the dye removal reaction, the solution was continuously stirred. For optical measurement of remaining MB dye, the dye was diluted (1/10 times) with de-ionized water. Each sample was taken at regular time intervals, and immediately centrifuged at 4000 rpm for 20 min to filter out photocatalyst particles and analyzed. Finally, the absorbance of the dye in the photocatalyst-filtered

supernatant was recorded at the maximum absorption wavelength of the dye using a UV-5100 spectrophotometer. The concentration of MB was calculated based on a calibration curve drawn before the experiment at a wavelength of 650 nm. The dye removal rate (η) is calculated as [64]:

$$\eta = (C_0 - C_t)/C_0 \times 100\% \quad (1)$$

where C_0 and C_t are the dye concentrations after self-photolysis and the different irradiation times, respectively.

Firstly, antibacterial activity was evaluated against the pathogen *Micrococcus luteus* and the marine bacterium *Bacillus manliponensis* as in Scheme 2c-1. The antibacterial activity of ZnOnanoplates was evaluated using Bauer Kirby's agar disk diffusion method. Luria-Bertani (LB) agar was weighed and dissolved in a warm water bath. Thereafter, it was sterilized in an autoclave at 120 °C and a pressure of 15 lbs for 15 min. After cooling the sterilized medium to 50 °C, 200 μ L of the medium was poured into each petri dish, sterilized under aseptic conditions, and allowed to solidify under laminar airflow. LB broth was inoculated with pure cultures of *Micrococcus luteus* and *Bacillus manliponensis* and stored for 24 h at 200 rpm on a rotary shaker at each 25 °C and 30 °C. The cell number was adjusted to $\sim 1 \times 10^6$ CFU/mL and plated onto LB agar plates. Discs of bacterial species containing particles of ZnO (10 mg) dissolved in water were placed on each plate and incubated at 37 °C. for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibitory (ZOI). The experiment was performed in triplicate and the ZOI diameter values were averaged. Second, as in Scheme 2c-2, to quantitatively analyze the effect of ZnO on overnight cultures on bacterial cells (typical gram-positive and gram-negative bacteria, *Staphylococcus aureus* and *Escherichia coli*), bacteria were inoculated on LB medium ($\sim 10^7$ CFU) with or without ZnO nanoplates. The culture flasks were then placed in a shaker incubator (200 rpm) for 0, 1, 2, or 3 h at 37 °C. In addition, the same operation was performed while irradiating LED (400 nm) light. After a certain period of time, samples were taken and serially diluted. Dilutions were spotted onto Muller Hinton Agar plates, and colonies were counted as CFU after 24 h of incubation. Inoculation-free medium containing no nanoparticles was used as a negative control. The bactericidal effect was calculated using the following formula [65]:

$$C(\%) = [(A - B)/A] \times 100\% \quad (2)$$

Here, C is the antibacterial and bactericidal effect, A is the average number of colonies formed in the control group (CFU/mL), and B is the average number of colonies formed in the experiment (CFU/mL).

4. Conclusions

In this study, we examined the relationship between oxygen vacancies in ZnO crystals and antibacterial performance. In order to express oxygen vacancies, four ZnOnanoplates were prepared by a hydrothermal process at different synthesis temperatures. These exhibited a nanoplate shape having a wurtzite crystal structure. As the crystallization temperature increased, the XRD peaks became sharper. Based on XPS, Raman, and PL spectra, it was predicted that the lower the crystallization temperature, the larger the defect in the crystal. The oxygen deficiency between the ZnO-50 and ZnO-200 nanoplates was small by about 1%, but the optical properties were significantly different. A ZnO-200 nanoplate with a few defects had the lowest PL intensity, but its photocurrent density was the highest, and it remained stable for 330 s. As a result, ZnO-200 nanoplates with a few vacancies show low electron recombination and easy charge transfer. Moreover, $\cdot\text{OH}$ and $\cdot\text{O}^{2-}$ radicals generated on the surface of ZnO were generated more in ZnO-200 nanoplates with fewer vacancies than in ZnO-50 nanoplates with many vacancies. In the end, this study concluded that oxygen vacancy in ZnO nanoplates with appropriate oxygen vacancies acted as an electron capture role, favoring charge transfer, and suppressing exciton recombination, resulting in excellent antibacterial and photoactive performance.

However, even in this study, it was difficult to determine with certainty how much of the oxygen vacancies in the crystal were appropriate and further research will be needed.

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