

EFFECT OF SILVER-MODIFICATION OF G-C₃N₄ ON PHOTOCATALYTIC DEGRADATION OF PARA-NITROPHENOL AND ANTIMICROBIAL ACTIVITY

XUAN TRUONG NGUYEN¹, TOAN VAN DUONG¹, LONG HOANG PHAM¹, LINH LE THI DIEU¹, GIANG TRUONG NGUYEN¹, PHONG QUOC TRUONG¹, CSABA ILCSIK² AND OTTÓ HORVÁTH^{3*}

¹ School of Chemistry and Life Sciences, Hanoi University of Science and Technology, No.1 Dai Co Viet Street, Bach Mai Ward, Hanoi, 100000, VIETNAM

² KNOT Kft., Bogáncs u. 27, Budapest, 1151, HUNGARY

³ Center for Natural Sciences, Faculty of Engineering, University of Pannonia, Egyetem u. 10, Veszprém, 8200, HUNGARY

Graphitic carbon nitride (g-C₃N₄) exhibits promising photocatalytic and antibacterial properties. This study aimed to enhance these activities via silver modification and to test its effects on the removal of para-nitrophenol (4-NP), a frequent aromatic water pollutant, and some dangerous bacteria under visible-light irradiation. The structural and optical properties of the silverized catalysts were characterized using XRD, SEM, FTIR, and UV-Vis-DRS techniques. Silver modification led to a ca. 50-fold increase in the photocatalytic degradation of 4-NP through better light absorption and hindered charge recombination. Scavenging experiments proved that 'O₂' and 'OH' species play a decisive role in this process. The presence of H₂O₂ contributed dramatically to the efficiency of decomposition through a synergistic effect. The individual decomposition rates were two orders of magnitude lower than the result achieved with the combined application of Ag/g-C₃N₄ and H₂O₂, primarily due to the increased 'OH' formation. The degradation conditions were optimized in terms of component concentrations. Since the surface charge of the Ag/g-C₃N₄ catalyst is zero at pH 6.76 (pH_{ZPC}), basic systems diminished the degradation efficiency due to repulsion between the negatively charged catalyst surface and deprotonated 4-NP. Although the antibacterial activity of Ag/g-C₃N₄ was not negligible against *Escherichia coli* and *S. aureus* in the dark, it became significantly enhanced under visible LED light or natural sunlight, photogenerating oxidative species that damage the cell wall. Therefore, the silver-doped g-C₃N₄ photocatalyst can be used efficiently for both the degradation of aromatic organic pollutants and the inactivation of dangerous bacteria in contaminated aqueous systems.

Keywords: photocatalytic degradation, para-nitrophenol, silver doping, disinfection, water purification

1. Introduction

Para-nitrophenol (4-NP) is an intermediate in the production of various pesticides and insecticides [1]. It is widely used in the chemical, pharmaceutical, and other industries, which has contributed to its status as an environmental pollutant [2],[3]. It is harmful to both animals and humans, causing cyanosis and methemoglobinemia, as well as damage to the liver, kidneys, and central nervous system [4]. Therefore, environmental regulations governing it are stringent; for example, according to the United States Environmental Protection Agency (EPA) regulations, the concentration of 4-NP in drinking water and wastewater must not exceed 0.43 µM [5] and 0.14 µM [6], respectively. Due to its high chemical stability and significant water solubility, its removal/disposal is not an easy task. Therefore, 4-NP has been designated as a priority

pollutant by the U.S. Environmental Protection Agency (EPA) [7]. Traditional treatment methods are often unsuitable for their disposal because they are either inefficient, too selective, or expensive [8]-[10]. Advanced oxidation processes (AOPs) offer a solution to these disadvantages, and among them, photocatalytic processes have recently received particular attention due to their advantages, such as high efficiency, low selectivity, environmentally friendly operation, and generally at low cost [9,11,12]. The latter properties are particularly evident when the photocatalyst effectively utilizes not only UV radiation but also visible light. An example of this is the photoactive semiconductor graphitic carbon nitride [13],[14], which has a relatively narrow band gap (2.7 eV) and can decompose various pollutants primarily through oxidation [15]-[17].

In addition to man-made organic compounds, various pathogens and epidemic-causing bacteria often

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*Correspondence: horvath.otto@mk.uni-pannon.hu

enter environmental waters, and their removal is also an important task for both nature conservation and the preparation of water for human consumption, e.g., drinking water. For instance, *Escherichia coli* (*E. coli*), a water-borne bacterium, can cause several intestinal and extra-intestinal illnesses, including septicemia, diarrhea, neonatal meningitis, and urinary tract infections [18]. Water resources exposed to waste discharge are likely to be contaminated by *E. coli*, with concentrations of up to 2×10^6 colony-forming units (CFU) per 100 mL of water [19]. *Staphylococcus aureus* (*S. aureus*) is another type of bacteria that causes serious infections in humans and animals [20]. These antimicrobial resistance (AMR) bacteria can enter the environment via various community and hospital wastewater discharges [21],[22]. Hence, the efficient disinfection of these wastewaters is crucial for our health and living environment. Photoactive semiconductors such as graphite nitride can also be used for such purification tasks through photogenerated oxidative particles [23]-[27]. In several cases, such catalysts have shown antibacterial effects even in the dark as a result of physical or chemical interactions [26,28,29]. It should be noted that the release of g-C₃N₄ into environmental waters may increase the tolerance of certain pathogens to antibiotics [30].

Graphitic carbon nitride (g-C₃N₄) is a tris-triazine-linked polymer consisting of carbon (C) and nitrogen (N) that can be readily synthesized [31],[32]. Several methods can be used for this purpose, e.g., physical vapor deposition [33], chemical vapor deposition [34], solvent-based thermal synthesis [35], and thermal polymerization [36]. Various precursors can be used for the latter process, e.g., dicyandiamide [37], carbamide [38],[39], or melamine [40]. A significant part of the research on g-C₃N₄ has focused on the oxidative degradation of organic pollutants, e.g., various dyes such as methyl red [41], and pharmaceuticals, antibiotics such as tetracycline or ciprofloxacin [42],[43], but photocatalytic reductions have also been realized [44]-[48]. Although the band gap of g-C₃N₄ is relatively narrow, it absorbs little in the visible range. Therefore, various methods have been explored to extend its absorption efficiency towards longer wavelengths, including (1) distorting the planar structure, (2) tuning the C/N ratio of g-C₃N₄, (3) inducing nitrogen vacancies, (4) improving the degree of polymerization, and (5) molecular and elemental doping [49].

The latter possibility can be realized with non-metallic or metallic elements [50]. In the case of metals, the plasmonic effect can promote the red shift of the absorption edge beyond 460 nm. Among several metals, silver offers a versatile option for increasing the light-harvesting efficiency and, thus, the photocatalytic activity of g-C₃N₄, mostly for the degradation of organic dyes such as methylene blue, methyl orange, Rhodamine B, or malachite green [51]-[55]. Nevertheless, colorless organic pollutants have also been degraded with Ag-g-C₃N₄ [56].

As mentioned above, g-C₃N₄ can also be used to neutralize waterborne pathogens and bacteria, which can be significantly enhanced by silverization of this catalyst.

Therefore, such a composite in a hydrogel exhibited promising photocatalytic antibacterial activity for wound dressing [57]. Notably, the antibacterial activity of Ag-doped g-C₃N₄ was also observed without photoexcitation [58].

Based on the previous results, our work aims to produce and test silver-modified g-C₃N₄ that is capable of photocatalytically decomposing para-nitrophenol, a colorless organic water pollutant, upon visible-light irradiation, while also being suitable for the elimination of various dangerous bacteria in the dark, and, especially, under the effect of photoexcitation.

2. Experimental

2.1. Reagents

Thiourea (CH₄N₂S, 99%), silver nitrate (AgNO₃, 99.8%) were purchased from Merck, Darmstadt, Germany. Sodium borohydride (NaBH₄, 98%), ethanol (C₂H₅OH, 99.7%), sodium hydroxide (NaOH, 99%), and hydrochloric acid (HCl, 36-38%) were obtained from GHTech, Guangzhou, China; 4-nitrophenol (C₆H₅NO₃, 99% from Macklin, Shanghai, China). All of these reagents were used without purification, and distilled water was prepared in the laboratory.

2.2. Synthesis of graphitic carbon nitride (g-C₃N₄)

The g-C₃N₄ was synthesized using a simple calcination method, using thiourea as a precursor. Typically, 3 g of thiourea were placed in a crucible with a cover. The calcination process was conducted at 550 °C for 2 hours, with a heating rate of 15 °C min⁻¹ in air. After calcination, the material was allowed to cool to room temperature. The sample was washed with distilled water and ethanol several times, then dried in an oven at 60 °C for 24 hours.

2.3. Synthesis of Ag/g-C₃N₄ composite

Typically, 0.5 g g-C₃N₄ was dispersed in 20 mL of distilled water by sonication for 10 min. Next, AgNO₃ (10 mL, 2.44 g/L) was added to the initial suspension and stirred in the dark for 1 hour. Then NaBH₄ was added (10 mL, 1 g/L) and stirred under light for 1 hour. Subsequently, the obtained precipitate was washed thoroughly with distilled water and ethanol to remove impurities and finally dried in an oven at 60 °C for 16 hours. The sample was denoted as 3wt% Ag/g-C₃N₄. To study the effect of Ag doping on the photocatalytic efficiency, the silver contents of 1, 3, 5, and 7 wt% were investigated.

2.4. Sample characterization

The prepared samples were characterized by scanning electron spectroscopy (SEM, NanoSEM 450FEI), energy dispersive X-ray (EDX, Super X), X-ray diffraction (XRD, Malvern PANalytical, Aeris), Fourier transform

infrared spectroscopy (FT-IR, Shimadzu, Irtaffinity-1S), diffuse reflectance UV-Visible spectroscopy (DR/UV-Vis, Carry 500 UV-Vis-NIR), and UV-Vis spectrophotometry (Agilent 8453, Santa Clara, CA, USA). Nitrogen sorption measurements for BET analysis were accomplished at 77.38 K on a Micromeritics ASAP 2060. Before the measurements, the samples were degassed under vacuum at 100 °C overnight.

To determine the point of zero charge and surface charge values, the pH drift method was applied [59],[60]. The 4-NP solution (100 mL, 10 mg/L) was adjusted to pH 2, 4, 6, 8, 10, and 12 at room temperature using 0.1 M NaOH and HCl, serving as the initial pH. Afterward, 70 mg of Ag/g-C₃N₄ photocatalyst was added, and the mixture was stirred in the dark for 24 hours. The suspension was then removed, and the final pH was measured.

2.5. Photocatalytic degradation experiments

The photocatalytic performance of the catalysts was evaluated by their efficiency in degrading 4-NP under irradiation from a 500-W Hg lamp simulating sunlight. The selected catalyst dosage was added to a solution containing 4-NP (at the chosen concentration, 100 mL) at an appropriate pH (using 0.1 M HCl or 0.1 M NaOH solutions) and then ultrasonicated for 3 min. The reaction beaker was stirred in the dark to obtain an adsorption-desorption equilibrium at room temperature. Then, an appropriate amount of H₂O₂ was added to the original beaker, and the mixture was exposed to light. At each time interval (10 min), 3 mL of the solution was collected and filtered through a 0.22 µm PTFE membrane to remove the catalyst. The degradation efficiency (DE) and the rate constant of 4-NP were calculated according to the following equations [61]:

$$DE (\%) = \frac{C_0 - C}{C_0} \quad (1),$$

$$-\ln \frac{C}{C_0} = kt \quad (2),$$

where C is the solution concentration of 4-NP (mg/L) at time t ; C_0 is the initial solution concentration; k is the rate constant; and t is the reaction time.

To identify the main reactive species in the 4-NP degradation process, experiments were conducted by adding radical scavengers to the solution: IPA (isopropyl alcohol - scavenges $\cdot\text{OH}$), AA (ascorbic acid - scavenges $\cdot\text{O}_2^-$), and AO (ammonium oxalate - scavenges h^+).

To examine the reusability of the Ag/g-C₃N₄ composite, consecutive experiments for the degradation of 4-NP were conducted over three cycles. After each cycle, the catalyst was recovered through filtration, washed multiple times with distilled water and ethanol, and then dried at 60 °C for 16 hours.

2.6. Determination of initial bacterial concentration from serial dilutions

The initial concentration of bacterial cells (expressed in colony-forming units per milliliter, CFU/mL) was

determined using the serial dilution and plate count method. Briefly, a known volume of the bacterial suspension was subjected to a series of tenfold dilutions, using sterile saline solution. From each dilution, 0.1 mL was spread onto nutrient agar plates, using the spread plate technique under aseptic conditions. The inoculated plates were incubated at 37 °C for 24 hours. After incubation, colonies appearing on plates within the countable ranges (30–300 colonies) were enumerated.

The bacterial concentration (N) was calculated according to the following formula, by summing the colony counts across selected plates, dividing by the total plated volume and dilution factors, and taking the logarithmic average where appropriate:

$$N \text{ (CFU/mL)} = \frac{\sum a}{V \times (n_1 + 0.1 n_2) \times d} \quad (3),$$

where

- $\sum a$: total number of colonies counted on all retained plates,
- V : volume plated on each agar plate (in mL),
- d : dilution factor corresponding to the first retained dilution (for undiluted liquid samples, $d=1$),
- n_1 : number of plates used at the first selected dilution,
- n_2 : number of plates used at the next tenfold dilution.

2.7. Evaluation of antibacterial activity of photocatalysts

In this study, the colony-forming unit (CFU) counting method was employed because it provides direct quantification of viable bacterial cells remaining after treatment. Unlike optical density (OD) measurements, this technique is not affected by dead cells or turbidity from suspended particles. Moreover, since Ag/g-C₃N₄ is an insoluble solid material, the agar diffusion method (e.g., inhibition zone test) was deemed unsuitable, as the photocatalyst cannot diffuse effectively through the agar medium.

A bacterial suspension was prepared by diluting the bacterial culture to approximately 10³ CFU/mL in 0.8% NaCl solution. This suspension (1 mL) was then mixed with photocatalyst materials (g-C₃N₄ or Ag/g-C₃N₄) at concentrations of 20, 40, and 60 mg/mL⁻¹. The mixtures were subsequently exposed to three different lighting conditions: 35W blue LED illumination, natural sunlight, and complete darkness. Each reaction was carried out at room temperature and conducted over two time periods: 30 minutes and 60 minutes. Before incubation, the suspensions were vortexed thoroughly to ensure uniform dispersion of the photocatalyst. After exposure, a 100 µL aliquot of each sample was plated onto nutrient agar, using the spread plate technique under sterile conditions, and incubated at 37 °C for 24 hours. After incubation, bacterial viability was evaluated by counting colony-forming units (CFUs). The antibacterial efficacy of each sample was quantified based on the bacterial counts using Equation 3. The percentage of bacterial

inactivation (i.e., antibacterial efficiency) under each experimental condition was then determined using the following equation.

$$\text{Antibacterial efficiency (\%)} = \frac{\text{Initial bacterial count} - \text{Remaining viable count}}{\text{Absorbance of control}} \times 100 \quad (4).$$

3. Results and discussion

3.1. Morphological and structural characterization

3.1.1. SEM analysis

The morphology of g-C₃N₄ (Figure 1a) is characterized by large sheet-like layers (2D structure) stacked upon one another, forming irregular blocks (3D structure) of varying sizes [62]. Following Ag doping, the surface of the doped g-C₃N₄ material (Figure 1b) exhibits increased roughness. This phenomenon can be attributed to the agglomeration of silver particles on the g-C₃N₄ surface. These aggregates cannot be observed due to their nanoscale size, which makes them difficult to detect at this magnification.

Figure S1 (in Supplementary Information) presents the EDX analysis results of the Ag/g-C₃N₄ composite. The findings confirm the successful Ag doping into the g-C₃N₄, as evidenced by the presence of Ag alongside C and N. The measured Ag content of 7.2wt%, which exceeds the initial doping level of 3wt%, suggests an uneven distribution of Ag on the g-C₃N₄ surface. Additionally, the absence of detectable impurities indicates the purity of the material.

3.1.2. BET analysis

The BET surface areas of g-C₃N₄ and 3wt% Ag/g-C₃N₄ were determined to be 46.45 m²/g and 44.07 m²/g, respectively. The corresponding adsorption-desorption plots are shown in Figure S2. These results indicate that silverization only slightly decreased the surface area of the catalyst. This decrease may be attributed to silver nanoparticles located in the surface pores of the catalyst, thereby reducing the accessible internal surface area.

3.1.3. XRD analysis

The XRD spectra of the g-C₃N₄ and Ag/g-C₃N₄ samples are presented in Figure 2. The g-C₃N₄ sample exhibits two characteristic diffraction peaks at 13.1° and 27.5°, corresponding to the (100) and (002) crystal planes, respectively (JCPDS No. 87-1526) [63],[64]. The peak at 13.1° is associated with the periodic arrangement of heptazine units, while the peak at 27.5° reflects the stacking of aromatic conjugated systems. Notably, the Ag/g-C₃N₄ composite does not display any new diffraction peaks, indicating that Ag doping does not alter the crystal structure of g-C₃N₄. The absence of a distinct characteristic peak for silver in Ag/g-C₃N₄ indicates a small silver particle size, low silver loading, and good

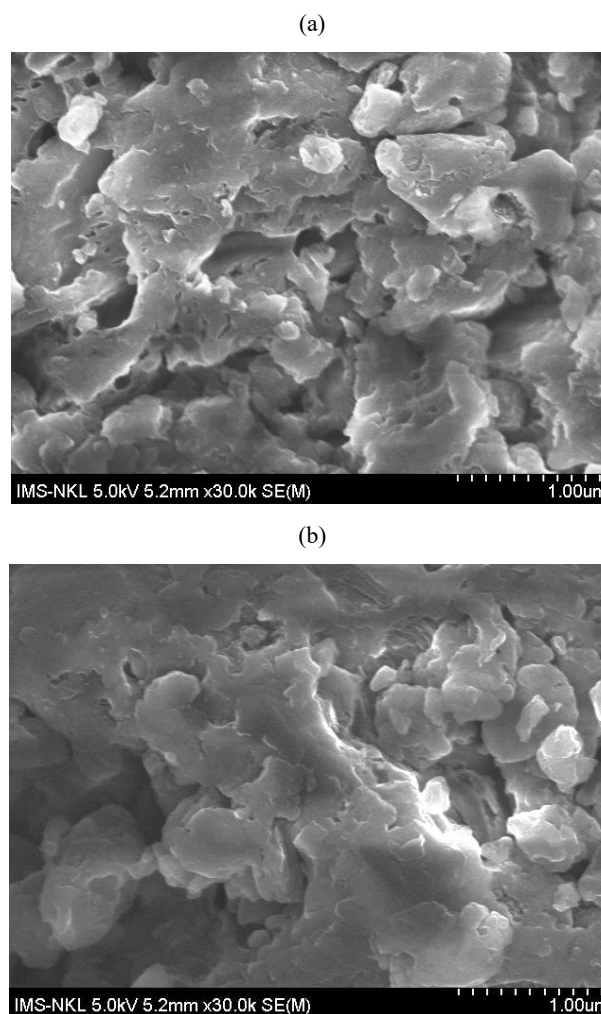


Figure 1: SEM images of (a) g-C₃N₄ and (b) 3wt% Ag/g-C₃N₄ samples

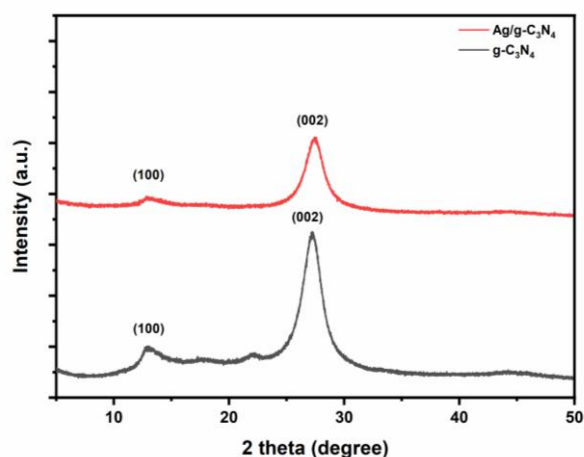


Figure 2: XRD spectra of g-C₃N₄ and 3wt% Ag/g-C₃N₄ samples

dispersion of the silver particles across the surface of g-C₃N₄. Furthermore, the absence of additional peaks

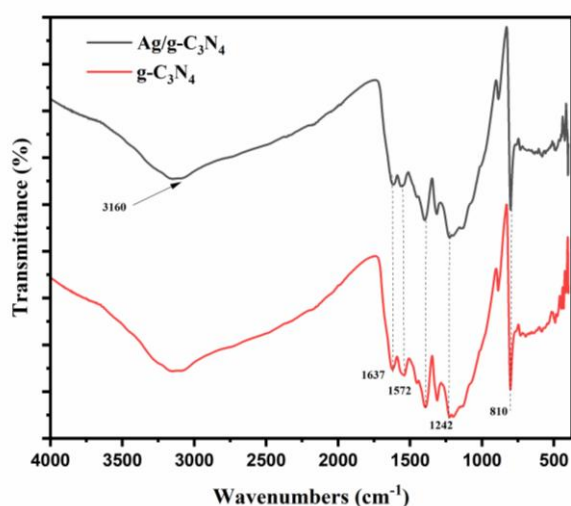


Figure 3: FT-IR spectra of g-C₃N₄ and 3wt% Ag/g-C₃N₄

suggests that the material remains uncontaminated and retains high purity.

3.1.4. FT-IR analysis

FT-IR spectra are shown in Figure 3. For g-C₃N₄, the peak at 810 cm⁻¹ corresponds to the out-of-plane deformation of the s-triazine unit [65]. Bands at 1572 cm⁻¹ and 1637 cm⁻¹ are attributed to C=N bond vibrations, while those between 1242 cm⁻¹ and 1409 cm⁻¹ correspond to C-N bond stretching in aromatic rings. The broad band at 3160 cm⁻¹ indicates stretching vibrations of -NH₂, =NH, or O-H from adsorbed water [66]. In addition, the absence of any absorption patterns associated with sulfur-containing bonds (such as -SH, -SN, and -SC) suggests that elemental sulfur has been fully released during the heat treatment process. The characteristic peaks of g-C₃N₄ remain unchanged after Ag doping, with no evidence of N-Ag bonding, suggesting that Ag is likely only deposited on the surface of g-C₃N₄ without forming covalent bonds [67].

3.1.5. DR/UV-Vis spectroscopic study

Figure 4a illustrates that both g-C₃N₄ and the Ag/g-C₃N₄ composite exhibit strong absorption in the 300–450 nm wavelength range. However, the Ag/g-C₃N₄ composite demonstrates significantly enhanced light absorption compared to pristine g-C₃N₄. In terms of band-gap energy (Figure 4b), g-C₃N₄ exhibits an absorption edge of approximately 460 nm, corresponding to a band gap of about 2.7 eV [68]. In contrast, the Ag/g-C₃N₄ composite reveals a reduced band-gap energy of 2.59 eV, as determined using the Tauc plot [54], with absorption extending to 480 nm. This reduction in band-gap energy is attributed to enhanced visible-light absorption facilitated by Ag NPs, which effectively utilize the surface plasmon resonance (SPR) effect. The observed shift in absorption from 460 nm to 480 nm indicates partial utilization of the visible region of the solar spectrum, which is advantageous for photocatalytic applications. The increased light absorption at longer wavelengths further confirms the successful integration of Ag NPs onto the g-C₃N₄ sheets, thereby enhancing the material's photocatalytic potential. The absence of a distinct absorption peak for Ag nanoparticles in the Ag/g-C₃N₄ composite can be explained by the relatively low silver content, which is insufficient to produce a characteristic absorption feature [64].

3.2. Effect of experimental conditions on the photocatalytic degradation of para-nitrophenol

3.2.1. Effect of Ag doping content

Upon irradiation of a reaction mixture containing the photocatalyst and 4-NP in the presence of H₂O₂, a gradual decrease in the absorbance of the 318-nm band of the substrate was observed (Figure S3). Preliminary experiments unambiguously indicated that silverization of g-C₃N₄ dramatically enhanced the degradation rate of 4-NP. Based on this observation, the effect of silver

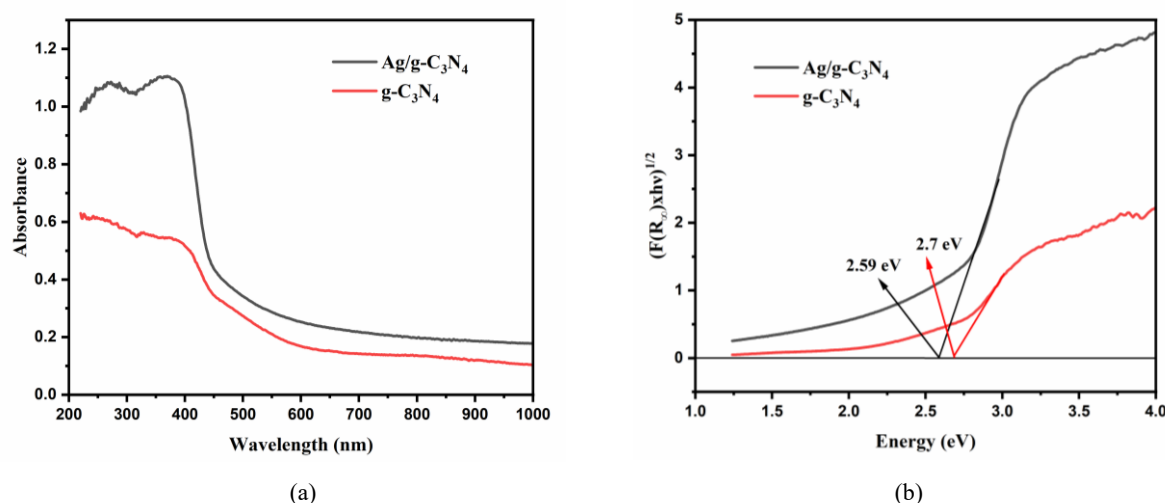


Figure 4: (a) DR/UV-Vis spectra and (b) Tauc plots of g-C₃N₄ and 3wt% Ag/g-C₃N₄ samples

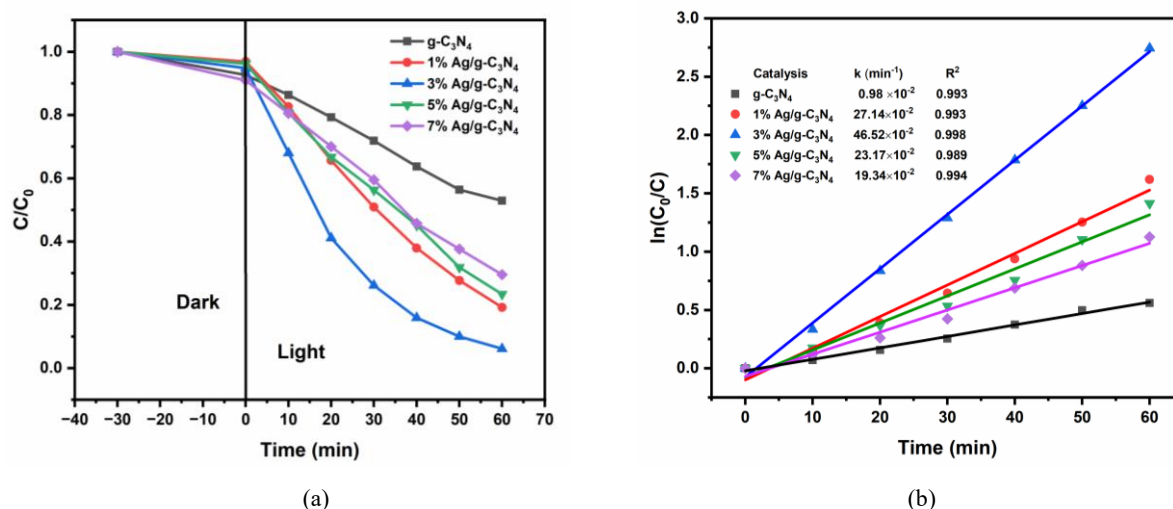


Figure 5: (a) Effect of Ag doping content on $g-C_3N_4$ and (b) kinetic curves.
Catalyst dose = 0.7 g/L, [4-NP] = 30 mg/L, $[H_2O_2]$ = 10 mM, pH = 3

doping on the degradation efficiency was further investigated.

Silverized photocatalysts were prepared with theoretical Ag contents of 1wt%, 3wt%, 5wt%, and 7wt%. Figure 5 illustrates that increasing silver content significantly improved the degradation of 4-NP. Specifically, within the doping range of 1wt% to 3wt%, the reaction rate constants were recorded at 27.14×10^{-2} and $46.52 \times 10^{-2} \text{ min}^{-1}$, respectively. This increase in photocatalytic efficiency with higher content of Ag NPs can be attributed to the surface plasmon resonance effect, which enhances the material's light absorption capabilities. Notably, at a 3wt% Ag doping level, the highest photocatalytic efficiency was achieved, reaching an impressive 94% after 60 minutes of irradiation. However, a further increase in Ag content led to a decline in reaction rates, with rate constants of only 23.17×10^{-2} and $19.34 \times 10^{-2} \text{ min}^{-1}$ observed at 5wt% and 7wt% doping levels, respectively. This phenomenon can be attributed

to the relatively high coverage of the catalyst surface by Ag NPs, which diminishes the efficiency of light absorption by the $g-C_3N_4$ catalyst. As the silver nanoparticles occupy a significant portion of the surface area, they obstruct the interaction between light and the catalyst, leading to reduced photocatalytic activity. Furthermore, this reduction in light absorption cannot be adequately compensated by the electron capture and transfer capabilities of the silver species, resulting in an overall decline in the photocatalytic performance of the system. Based on these results, the Ag/g- C_3N_4 catalyst obtained with 3wt% doping was applied for further studies.

3.2.2. Effect of initial solution pH

The degradation of 4-NP was studied over a pH range from 3 to 9. Figure 6 presents the rate constants at pH values of 3, 5, 7, and 9, which are 46.52×10^{-2} , 10.08×10^{-2} , 2.53×10^{-3} , and $1.77 \times 10^{-3} \text{ min}^{-1}$, respectively.

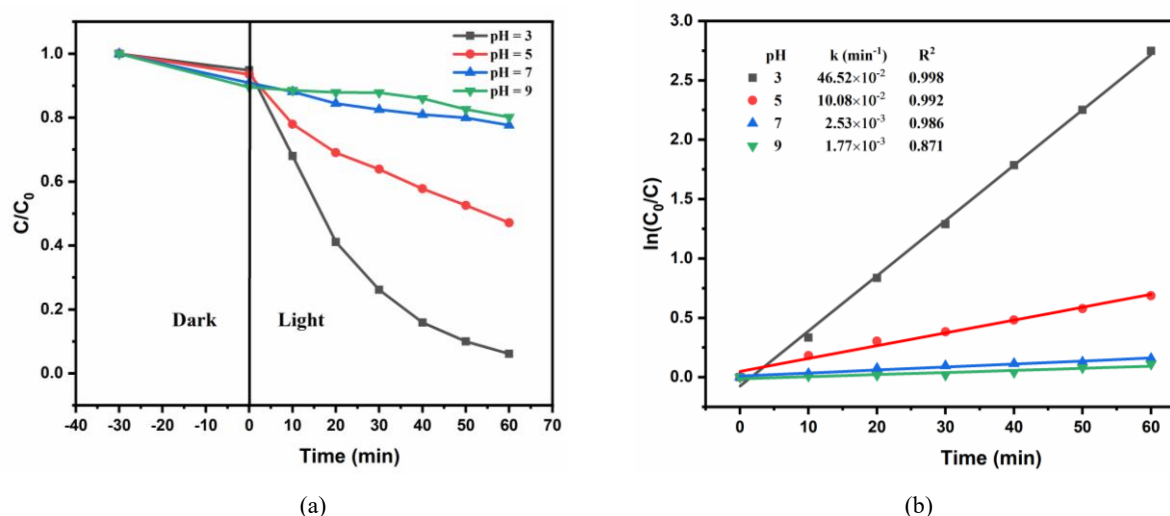
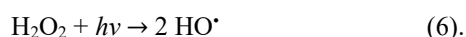


Figure 6: (a) Effect of pH on photocatalytic efficiency and (b) corresponding kinetic curves.
Catalyst dose = 0.7 g/L 3wt% Ag/g- C_3N_4 , [4-NP] = 30 mg/L, $[H_2O_2]$ = 10 mM

The photocatalytic degradation of organic pollutants by g-C₃N₄ in the presence of the strong oxidant H₂O₂ generates hydroxyl (HO[•]) radicals [69].



Photodissociation of hydrogen peroxide (Equation 6) does not play a significant role in this system because H₂O₂ absorbs only in the UV range. The rate of H₂O₂ reduction (Equation 5) is significantly influenced by pH. Basic systems facilitate the deprotonation of hydrogen peroxide, which is not favorable for the generation of hydroxyl radicals due to the repulsion between the negatively charged species. Accordingly, higher pH levels correlate with a reduction in the degradation rate of 4-NP. The pH of the solution has a significant influence on the surface charge of nanoparticles, thereby affecting the adsorption of organic pollutants. The point of zero charge (pH_{pzc}) is the pH at which the material's surface is neutral. When pH < pH_{pzc}, the catalyst surface is positively charged, enhancing anion adsorption. Conversely, when pH > pH_{pzc}, the surface becomes negatively charged, favoring cation adsorption. The pH_{pzc} of the Ag/g-C₃N₄ composite was determined to be 6.76 (Figure S4). This value is slightly lower than that reported for the metal-free g-C₃N₄ (6.97 [70]).

The presence of 4-NP is pH-dependent (Figure 7). Its pK value is 7.15 [71]. Hence, at pH values from 3 to 6, 4-NP exists in the protonated form. In the pH range of 7 to 11, it exists in the deprotonated form, which carries a negative charge. Consequently, at pH values of 7 and 9, the surface charge of the material and the charge of 4-NP are the same, leading to repulsive interactions that decrease treatment efficiency. In the pH range studied, the highest degradation rate was obtained at pH=3. Hence, further experiments were carried out at this pH.

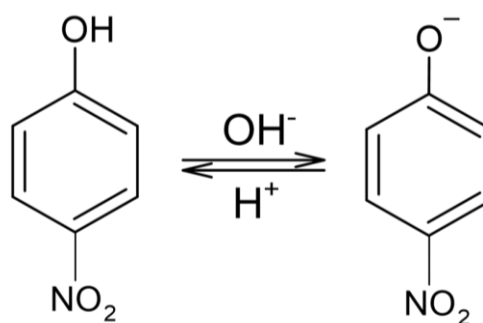


Figure 7: The formula of 4-NP depending on the pH environment

3.2.3. Effect of photocatalyst concentration

The influence of catalyst concentration (3wt% Ag/g-C₃N₄) was investigated by varying the concentration from 0.3 to 1.0 g/L under the same reaction conditions ([4-NP] = 30 mg/L, [H₂O₂] = 10 mM, pH = 3). Figure 8 shows that increasing the catalyst concentration enhances the degradation efficiency and reaction rate of 4-NP. The rate constants at 0.3 g/L and 0.5 g/L are 43.07 × 10⁻² and 56.12 × 10⁻² min⁻¹, respectively, with the highest degradation efficiency of 96.4% at 0.5 g/L. However, at 0.7 g/L, photocatalytic efficiency begins to decline, with a more pronounced decrease at 1.0 g/L, when rate constants were only 46.52 × 10⁻² and 28.46 × 10⁻² min⁻¹, respectively. This trend can be attributed to two factors: initially, increasing the catalyst amount enhances the number of active sites on the catalytic surface and increases the interaction density between light and the catalyst, promoting the generation of reactive radicals such as [•]O₂⁻ and HO[•], which effectively degrade organic pollutants. Conversely, excessive catalyst content leads to a turbid reaction

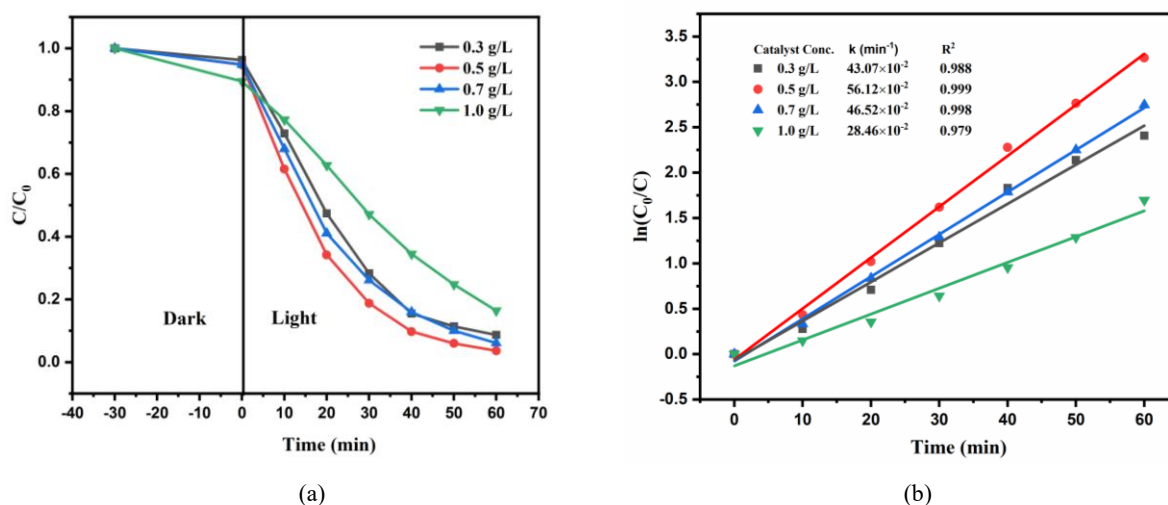


Figure 8: (a) Effect of catalyst concentration on the 4-NP degradation efficiency of 3wt% Ag/g-C₃N₄; (b) corresponding kinetic curves

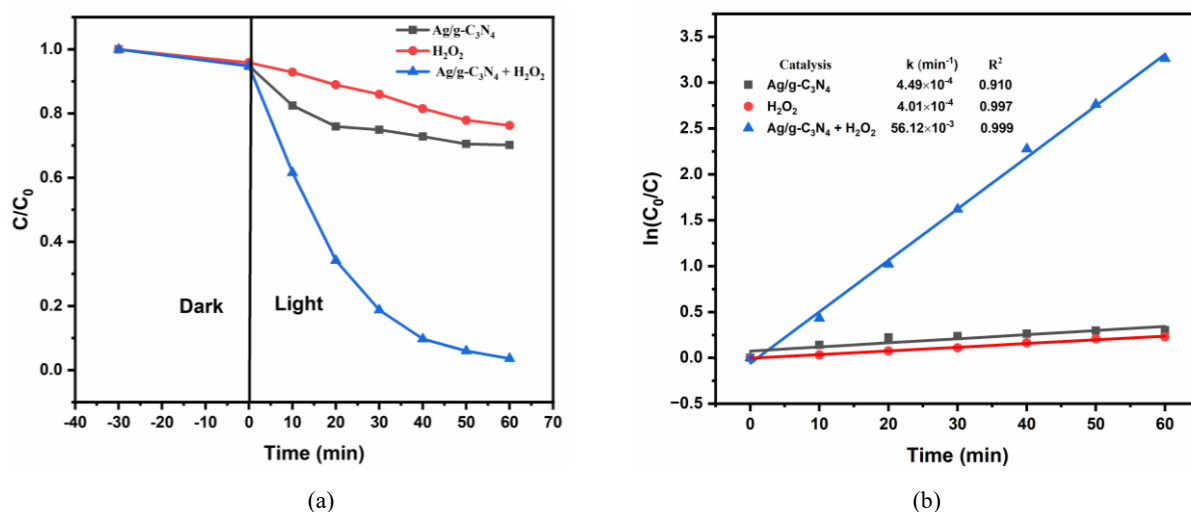


Figure 9: Photocatalytic performance of H_2O_2 , 3wt% $\text{Ag/g-C}_3\text{N}_4$, and 3wt% $\text{Ag/g-C}_3\text{N}_4 + \text{H}_2\text{O}_2$; (b) corresponding kinetic curves. Catalyst dose = 0.5 g/L, [4-NP] = 30 mg/L, $[\text{H}_2\text{O}_2]$ = 10 mM, pH = 3

environment, increasing the density of suspended particles, which reduces contact opportunities between the catalyst and pollutant molecules and hinders light penetration, thereby increasing light scattering effects [72]. Therefore, the optimal catalyst content was 0.5 g/L, and it was used for further investigations.

3.2.4. Effect of H_2O_2 concentration

Previous studies have indicated that treating 4-NP is challenging without strong oxidizing agents [73],[74]. To enhance the treatment capacity, a specific amount of H_2O_2 was added during the process (Figure 9). The presence of H_2O_2 in the photocatalytic reaction with $\text{Ag/g-C}_3\text{N}_4$ significantly improved the system's catalytic activity by generating hydroxyl radicals on the catalyst surface (see Equation 5). These radicals are potent oxidizing agents capable of attacking organic molecules that are difficult to decompose in aqueous environments. The degradation efficiency (DE) reached 96.4%, whereas using H_2O_2 alone resulted in a DE of only 23.7%,

indicating that H_2O_2 is not the primary degrading agent. Considering the degradation rate constants measured under various experimental conditions (Figure 9b), it is quite obvious that the combined application of the silverized photocatalyst and H_2O_2 resulted in a synergistic effect of almost two orders of magnitude. The sum of the two individual rate constants was less than $9 \times 10^{-4} \text{ min}^{-1}$, while that for the combined treatment reached $5.6 \times 10^{-2} \text{ min}^{-1}$. This dramatic effect can be attributed to the production of $\cdot\text{OH}$ radicals in the reaction between H_2O_2 and the photogenerated conduction-band electrons (Equation 5).

The H_2O_2 concentration is crucial for enhancing treatment capacity. Concentrations of 5 mM, 10 mM, 15 mM, and 20 mM yielded degradation efficiencies for 4-NP of 80.21%, 96.40%, 98.29%, and 99.54%, respectively. Results are illustrated in Figure 10, indicating that while increasing H_2O_2 concentration generally improves efficiency, the rate of improvement diminishes at higher concentrations. When the H_2O_2

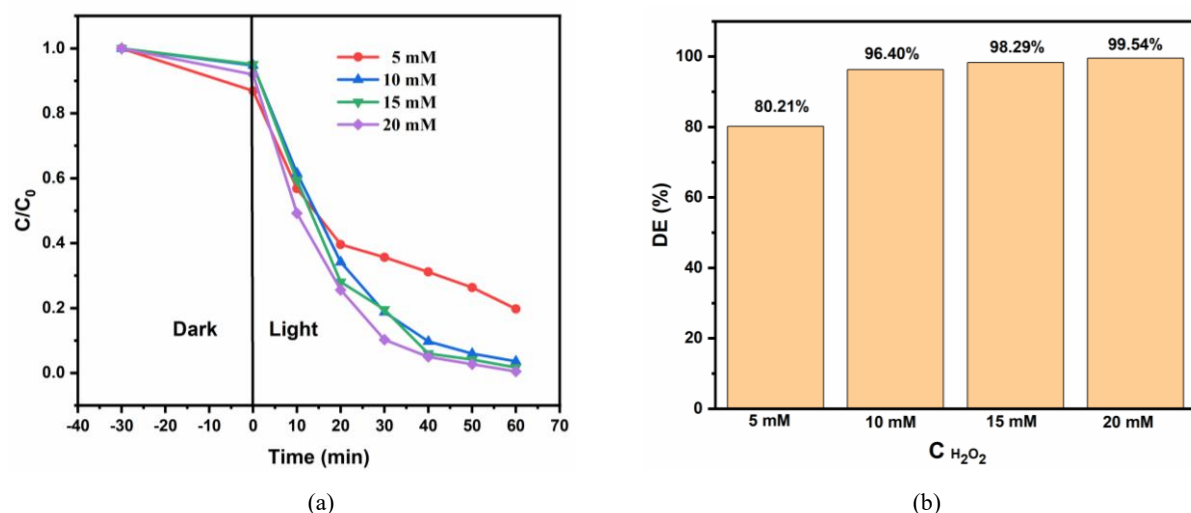


Figure 10: Effect of H_2O_2 concentration

concentration increased to 15 mM, the degradation efficiency did not change significantly, and compared with 10 mM, the *DE* was enhanced by less than 2%, from 96.4% to 98.29%. However, the excessive use of H₂O₂ affects both the economy and the environment. Consequently, the concentration of 10 mM was selected for further experiments.

3.2.5. Effect of initial 4-NP concentration

The effect of the 4-NP concentration was investigated in the range of 20 – 50 mg/L under the same reaction conditions (3wt% Ag/g-C₃N₄ catalyst dose = 0.5 g/L, [H₂O₂] = 10 mM, pH = 3). As presented in Figure 11, the rate constants for the degradation of 4-NP at initial concentrations of 20, 30, 40, and 50 mg/L were 79.07, 56.11, 24.26, and 13.04 ($\times 10^{-2}/\text{min}$), respectively. This tendency indicates that increasing the initial concentration of 4-NP decreased the degradation rate constant. However, the absolute degradation rate (i.e., the product of the rate constant and the initial 4-NP concentration) increased moderately as the concentration increased from 20 to 30 mg/L. Nevertheless, the further increase of the 4-NP concentration resulted in a gradual and significant decrease in the absolute degradation rate, too. This decline in the rate can be attributed to two factors: (1) As the number of 4-NP molecules rises, the adsorption of 4-NP on the active sites also increases, which can hinder the absorption of the incident photons by the catalyst. Consequently, the rate of generation of HO[•] and [•]O₂⁻ decreases. (2) As the 4-NP concentration increases, the number of intermediates also rises, creating competition with 4-NP molecules for the catalyst's active sites, which reduces photocatalytic activity, further diminishing photocatalytic efficiency [75]. Based on these results, a concentration of 30 mg/L was selected for further studies.

3.3. Mechanism of the photocatalytic degradation of 4-nitrophenol with Ag/g-C₃N₄

To clarify the role of active species in 4-NP degradation, radical trapping experiments were conducted. The results of these experiments are presented in Figure 12.

The degradation efficiency of 4-NP decreased from 96.4% to 84.7% when AO (ammonium oxalate, a hole scavenger) was added. This efficiency decreased even more significantly when scavengers such as IPA (isopropyl alcohol, a scavenger of HO[•]) or AA (ascorbic acid, a scavenger of [•]O₂⁻) were added, with the remaining efficiencies being 21.3% and 17.5%, respectively. This result suggests that [•]OH and [•]O₂⁻ radicals act as the two main agents in the degradation of 4-NP.

The conduction band (CB) and valence band (VB) values of Ag/g-C₃N₄ are determined as $E_{\text{CB}} = -1.13$ eV and $E_{\text{VB}} = +1.47$ eV [69]. When the Ag/g-C₃N₄ composite is irradiated by a light source of energy equal to or greater than its band-gap energy, electrons (e⁻) in the VB are excited and transferred to the CB, leaving behind holes (h⁺) in the VB. The VB potential is +1.47 eV, which is more negative than the standard potential of the [•]OH/H₂O pair (+2.40 eV) [61], indicating that h⁺ cannot oxidize water molecules to generate HO[•] radicals. However, it is more positive than the standard potential of the H₂O/O₂ pair (1.23 eV) [76], allowing the holes to oxidize water (H₂O) to produce protons (H⁺) and O₂. Additionally, the CB potential is -1.13 eV, which is more negative than the redox potential of the O₂/[•]O₂⁻ pair (-0.33 eV) [77], facilitating the reaction of electrons with O₂ to generate [•]O₂⁻ radicals, one of the two main reagents in the reaction. In radical trapping experiments, HO[•] also serves as a key reagent. This is explained by the fact that the generated [•]O₂⁻ can further interact with H₂O on the material surface, leading to the formation of hydrogen peroxide (H₂O₂). H₂O₂ can react with e⁻ or [•]O₂⁻ radicals, or even light photons, resulting in the production of [•]OH radicals. H₂O₂ significantly enhances the oxidation

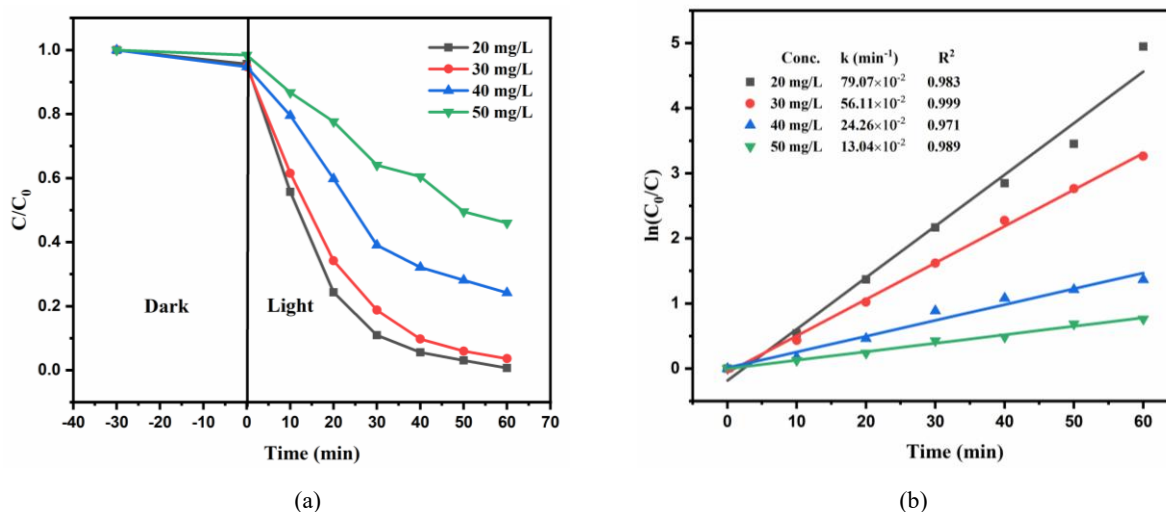


Figure 11: (a) Effect of 4-NP concentration on catalytic efficiency and (b) kinetic curves

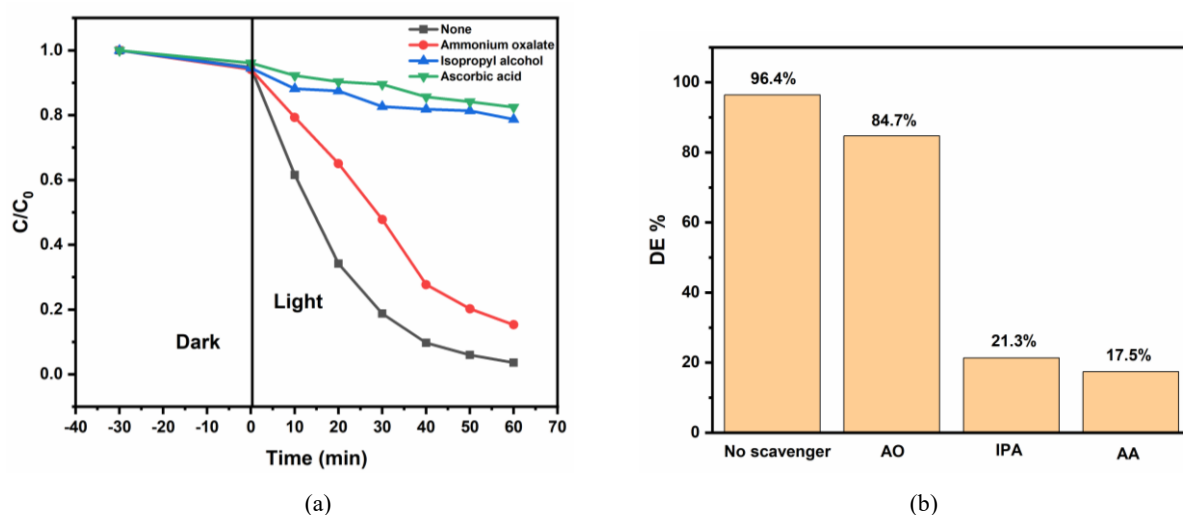
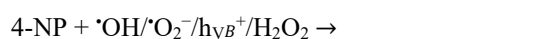
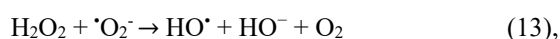
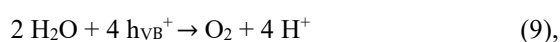
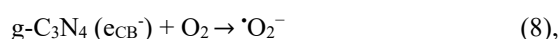


Figure 12: Photocatalytic efficiency of 4-NP, using radical scavengers

process by contributing to the generation of hydroxyl radicals, thereby accelerating the photocatalytic reaction. The following equations, completed with Equations 5-6, summarize the reaction steps playing important roles in the photocatalytic oxidative degradation of 4-NP, while Figure S5 provides a simplified mechanism of the photocatalytic degradation of 4-NP.



3.4. Reusability of the photocatalyst

The reusability of a material is a critical parameter for assessing its photocatalytic activity. In this study, the photocatalytic performance of the Ag/g-C₃N₄ composite was evaluated over three consecutive cycles, as depicted in Figure S6. The initial photocatalytic efficiency was 96.40%, decreasing to 85.56% after three reuse cycles, indicating the material's effectiveness in degrading 4-NP. The observed decline in performance may be attributed to the catalyst loss during the collection process after each irradiation cycle, underscoring the importance of optimizing the recovery methods to enhance the stability and reusability of the photocatalyst.

3.5. Antibacterial activity of Ag-g-C₃N₄

As mentioned in the Introduction, silverized graphitic carbon nitride is expected to provide antibacterial effects,

partly due to its Ag content, which may be harmful to various bacteria even in the dark, partly because of its capability for photogeneration of oxidative species destroying the cell walls of the microbes.

3.5.1. Antibacterial activity against *E. coli*

Figure 13a illustrates the antibacterial performance of g-C₃N₄ and Ag/g-C₃N₄ against *E. coli* at different initial photocatalyst concentrations (20, 40, and 60 mg/mL) and various light conditions over a 30-minute exposure period. The results indicate that g-C₃N₄ alone exhibited negligible antibacterial activity in the absence of light, with 100% bacterial survival. Under light irradiation, its performance improved slightly, reaching a maximum of ~15% under sunlight and ~20% under LED light at the highest concentration, suggesting limited photocatalytic effectiveness. In contrast, Ag/g-C₃N₄ demonstrated significantly enhanced antibacterial activity, particularly under LED illumination, achieving complete bacterial inactivation (100%) at 60 mg/mL. A clear concentration-dependent trend was observed, with antibacterial efficiency increasing with higher photocatalyst dosage across all lighting conditions. Notably, even in the absence of light, Ag/g-C₃N₄ retained moderate antibacterial activity (36%), highlighting the intrinsic antimicrobial role of silver. At 20 mg/mL, the effect of light was evident: under LED light, the bacterial reduction was 67%, nearly double that observed in the dark (36%), confirming the role of photocatalysis. At 60 mg/mL, both LED and sunlight conditions resulted in complete bacterial inhibition, suggesting a saturation threshold for effective antibacterial action.

Figure 13b shows the antibacterial efficiency of the materials over 60 minutes at varying concentrations. Under dark conditions, g-C₃N₄ showed no antibacterial effect; bacterial growth even increased, confirming its inactivity in the absence of light. Even at higher concentrations, g-C₃N₄ remained ineffective without photocatalytic activation. Under visible light, its performance improved slightly (up to ~13%), limited by fast electron-hole recombination and low visible-light

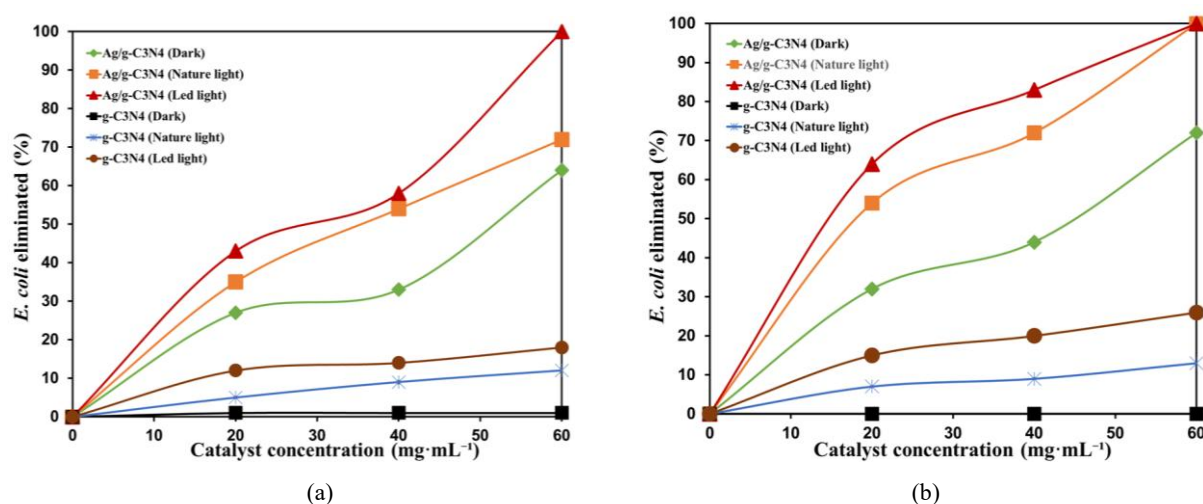


Figure 13: Antibacterial activity against *E. coli* by Ag/g-C₃N₄ and g-C₃N₄ at different concentrations under (a) 30-minute and (b) 60-minute light exposure

absorption. In contrast, Ag/g-C₃N₄ showed strong antibacterial activity across all conditions. At 60 mg/mL, complete bacterial elimination (100%) was achieved under both LED and natural light. Even at 20 mg/mL, the material reduced bacterial viability by ~55% under visible light and ~70% under LED. These results confirm that increasing both the photocatalyst dose and exposure time enhances antibacterial performance. The material is particularly effective against *E. coli*, suggesting its potential for antibacterial applications against Gram-negative bacteria.

3.5.2. Antibacterial activity against *S. aureus*

Figure 14 shows that *S. aureus* was less susceptible to both g-C₃N₄ and Ag/g-C₃N₄ compared to *E. coli*, especially under natural light and in the dark. This is likely due to the thick peptidoglycan layer in Gram-positive bacteria, which reduces surface adhesion and ROS penetration. g-C₃N₄ showed no antibacterial effect in the absence of light and only limited activity under

visible (~10%) and LED light (~15%), even at 60 mg/mL, due to poor quantum efficiency and rapid electron-hole recombination. In contrast, Ag/g-C₃N₄ exhibited improved antibacterial performance, increasing with both catalyst dose and light intensity. Under visible light, Ag/g-C₃N₄ reached 32% inhibition at 40 mg/mL and 43% at 60 mg/mL. LED irradiation further enhanced activity, with a maximum of 64%. Although overall performance was lower than that against *E. coli*, full inactivation (100%) of *S. aureus* was achieved at 60 mg/mL after 60 minutes under LED light, confirming strong photocatalytic antibacterial potential.

Figure 14b highlights the significant improvement in antibacterial performance when using Ag/g-C₃N₄ compared to pristine g-C₃N₄ against *S. aureus* under LED illumination. While g-C₃N₄ achieved only 26% inhibition at 60 mg/mL, Ag/g-C₃N₄ reached complete bacterial elimination (100%) under the same conditions—an increase of 74%, demonstrating the enhanced photocatalytic efficiency of the composite. Although silver alone exhibits inherent antibacterial activity, its

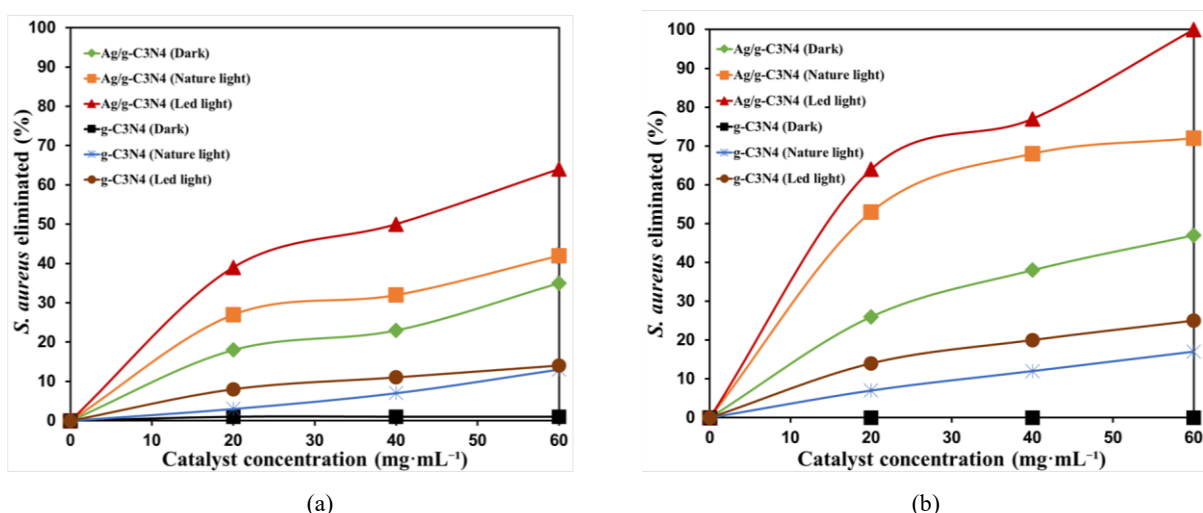


Figure 14: Antibacterial activity against *S. aureus* by Ag/g-C₃N₄ and g-C₃N₄ at different concentrations under (a) 30-minute and (b) 60-minute light exposure

contribution under dark conditions was limited to ~46% at the highest concentration, confirming that light-induced photocatalysis was the main driver of bacterial inactivation. The enhanced activity can be attributed to improved light absorption, suppressed electron–hole recombination, and increased ROS generation. *Figure S7* displays the bacterial growth under different treatment conditions. *Figure S7a* represents the control sample, in which bacteria were grown without any photocatalyst, demonstrating full bacterial proliferation. A clear trend of increasing antibacterial activity with increasing catalyst concentration was observed. At 60 mg/mL (*Figure S7d*), no visible bacterial colonies were detected, and the inhibition zone fully covered the agar surface, indicating 100% bacterial elimination. In contrast, at the lowest tested concentration of 20 mg/mL (*Figure S7b*), significant bacterial growth was still visible, suggesting limited inhibition at this dose. These visual results strongly support the quantitative findings, confirming the dose-dependent antibacterial performance of the Ag/g-C₃N₄ photocatalyst.

While the antibacterial effect against *S. aureus* (Gram-positive) was slightly lower than that against *E. coli* (Gram-negative)—72% vs. 100% under visible light at 60 mg/mL—Ag/g-C₃N₄ still showed a strong and consistent performance. The lower efficiency against *S. aureus* may be due to its thicker peptidoglycan cell wall, which provides greater resistance to oxidative damage. Overall, the results confirm that Ag/g-C₃N₄ is a promising visible-light-driven photocatalyst with effective antibacterial properties.

4. Conclusions

It has been demonstrated that the photocatalytic properties of g-C₃N₄ can be significantly improved by silver-doping. Although this resulted in a slight decrease in the band gap, manifested in a modest increase of light absorption in the visible range, the efficiency for the degradation of the water pollutant 4-NP was enhanced primarily through the suppression of charge recombination. The degradation efficacy was dramatically increased through the synergistic effect of H₂O₂, which strongly promoted the formation of •OH radicals that play an important role in the decomposition mechanism. The effects of the photocatalytic degradation conditions were interpreted and optimized.

The silverized catalyst also exhibited significant antimicrobial activity against pathogenic bacteria. The antibacterial effect was considerably enhanced by irradiation (with visible LED and natural sunlight), suggesting the significant role of the photogenerated oxidative species in damaging the cell wall.

These results clearly demonstrate that Ag-g-C₃N₄ offers a promising tool for the effective degradation of aromatic organic pollutants and, simultaneously, for the inactivation of pathogenic bacteria in various types of wastewaters.

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Supplementary information

The supplementary material is available from the editorial office and can be provided upon request.

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