Enhanced visible-light-driven photodegradation of tetracycline by salicylic acid modified-graphitic carbon nitride and toxicity assessment

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Abstract

The tetracycline (TC) in water has led to serious concern. Graphitic carbon nitride (g-C$_3$N$_4$) photocatalysts were produced via copolymerization of mono-benzene ring-mediated precursors (urea, melamine, and dicyandiamide) involving salicylic acid (SA) for TC degradation. The SA-modified g-C$_3$N$_4$ samples showed improved visible-light absorbance, transfer and separation of photogenerated electrons, and prospective photocatalytic application in TC degradation. As a result, the optimal SA-modified g-C$_3$N$_4$ (2 wt% of SA) using urea (CNU-SA-2) showed 2 times higher TC degradation than that of pristine g-C$_3$N$_4$. The process of TC degradation was evaluated by the reduction of antibacterial activity and extensively studied by varying the types of TC, initial pH values, co-existing anions, and natural organic materials. In addition, the catalyst could be reused for at least four cycles, indicating good reusability. The main active species were revealed to be h$^+$ and ·O$_2^-$ by scavenging experiments and electron spin resonance. The CNU-SA-2 photocatalyst and TC intermediates during degradation had no adverse impact on zebrafish embryos. This work could provide a design strategy and a perspective on the practical application of g-C$_3$N$_4$-based photocatalysts for the treatment of wastewater containing antibiotics.

Introduction

Antibiotics have been extensively used in human health, animal husbandry, and agriculture. The existence of antibiotics in aquatic environments leads to the development of antibiotic-resistant bacteria and genes, posing threats to organisms and humans (Xu et al. 2021). Tetracycline (TC), as a typical antibiotic, is frequently detected in water (Wei et al. 2010). Conventional wastewater treatment approaches, such as filtration, adsorption, and biodegradation cannot completely degrade TC because of the naphthalene ring structure and hydrophilic property (Watkinson et al. 2007). Recently, solar light-driven nanomaterial-based semiconductor photocatalysis is a promising strategy due to its high efficiency, easy operation, high mineralization rate, and environmental compatibility (Li et al. 2022, Lin et al. 2021).

Among the semiconductor photocatalysts, metal-free graphitic carbon nitride (g-C$_3$N$_4$) is a highly expected candidate for wastewater remediation. g-C$_3$N$_4$ can be prepared using a simple thermal condensation with economic precursors (urea, melamine, and dicyandiamide). g-C$_3$N$_4$ possesses high physicochemical stability as well as unique optical and electronic properties (Zhu et al. 2020). However, the ineffective light absorption and fast photogenerated charge recombination of g-C$_3$N$_4$ restrict its practical application (Liu et al. 2019a, Zhou et al. 2019b). To address the limitations, several efforts including nanostructure design (Liu et al. 2019b, Xia et al. 2017), heterojunctions with semiconductors, and copolymerization (Zhang et al. 2012) have been conducted. The copolymerization of g-C$_3$N$_4$ precursors with non-metal aromatic structure is a promising technique since metal leaching into the aquatic environment, especially for toxic ones like Ag$^+$, Co$^{2+}$, Zn$^{2+}$, etc., can be avoided. Additionally, the aromatic structure is similar to that of the triazine phase of g-C$_3$N$_4$. Anchoring aromatic rings in the g-C$_3$N$_4$ network could regulate the intrinsic electronic property and manipulate the bandgap. The photocatalytic activity is consequently enhanced by increasing visible light harvesting and improving
charge pair separation. The photocatalytic performance of g-C\textsubscript{3}N\textsubscript{4} could be improved by copolymerizing organic acid (anthroic acid, benzoic acid, and naphthoic acid) with urea due to the fact that the benzene-ring in the g-C\textsubscript{3}N\textsubscript{4} framework could increase the visible light absorbance (Li et al. 2018). Salicylic acid (SA) has a benzene-based structure and was proved to improve photogenerated charge carrier transmission of g-C\textsubscript{3}N\textsubscript{4} nanotubes after the copolymerization with melamine (Jia et al. 2019). In previous studies, SA-modified g-C\textsubscript{3}N\textsubscript{4} showed excellent performance in the degradation of sulfamethazine and TC (Zhou et al. 2019a). However, it is not yet known if g-C\textsubscript{3}N\textsubscript{4} with benzene-ring synthesized using different precursors have optimized degradation activities. At the same time, the mechanism responsible for TC removal needs to be clarified.

On the other hand, in the process of degradation, the treatment of antibiotic-contaminated wastewater has recently raised new concerns because of the generation of high toxic intermediates (Osin et al. 2018, Zhao et al. 2020). For example, Han et al. reported that the toxicity of a tetracycline solution increased with the increased photocatalytic time and the intermediate (m/z 477.15) was deemed as the compound leading to the solution toxicity (Han et al. 2020). Additionally, Zhao et al. found that many products with more toxicity were generated during the degradation of tetracycline (Zhao et al. 2020). But, to the best of our knowledge, there are no reports to evaluate the possible toxicity of the transformation products during the degradation process (benzene-ring-modified g-C\textsubscript{3}N\textsubscript{4} system). Meanwhile, the increasing applications of g-C\textsubscript{3}N\textsubscript{4} photocatalysts have also triggered safety concerns (He et al. 2020, Lin et al. 2018). Their potential risks are seldomly reported. So, the ecotoxicity of the benzene-ring-modified g-C\textsubscript{3}N\textsubscript{4} photocatalysts, TC, and the intermediates needs assessment, which is a crucial factor of the potential application.

In this report, g-C\textsubscript{3}N\textsubscript{4} photocatalysts with benzene-ring were constructed by thermal copolymerization of salicylic acid (SA) with different precursors (urea, melamine, and dicyandiamide). The photocatalytic performance was investigated by TC degradation under visible LED light irradiation. The effects of the initial pH values, the co-existing ions, natural organic matter, and types of TCs were studied. Regeneration tests were investigated as well to assess the reusability. The mechanism of TC photodegradation was determined. The toxicity of the photocatalysts and TC degradation intermediates was evaluated using zebrafish embryo and/or bacteria inhibition tests. This study presents a reference for modifying g-C\textsubscript{3}N\textsubscript{4} with aromatic structure and facilitates the practical application of g-C\textsubscript{3}N\textsubscript{4}-based photocatalysts.

**Materials And Methods**

**Materials synthesis**

All the chemicals used in this study were analytical reagent grade, and detailed information was shown in Text S1.
The g-C$_3$N$_4$ samples (CNU, CNM, and CND) were prepared from urea, melamine, and dicyandiamide by heating at 550°C (5°C/min) in a muffle furnace for 2 h. The SA-modified CNU, CNM, and CND were synthesized by mixing 10 g of precursors (urea, melamine, and dicyandiamide) and fixed amounts of SA (0.05, 0.2, and 0.3 g) in 15 mL of 33% (V/V) ethanol solution for 2 h. Then, the mixed solutions were treated with ultrasonication for 0.5 h and then dried at 80 °C overnight. Subsequently, the dried mixtures were annealed at 550 °C (5°C/min) in a muffle furnace for 2 h. The obtained solids were ground into powder. The synthesized samples were denoted as CNU-SA-x, CNM-SA-x and CND-SA-x, where x represented the mass percentage of SA.

**Characterizations**

X-ray diffraction (XRD) with Cu Kα as the radiation source was used to study the crystal phase of the synthesized photocatalysts (Rigaku Ultimate IV, Japan). Fourier transform infrared spectroscopy (FT-IR) was conducted on a Thermo Scientific Nicolet iS5 FT-IR spectrometer between wavenumbers of 4000 and 400 cm$^{-1}$. The detailed morphologies of the samples were observed using scanning electron microscopy (SEM) (Zeiss, Sigma 300, Germany). The specific surface areas and pore volume of the photocatalysts were determined by the Brunner-Emmet-Teller (BET) analysis (ASAP2460, USA). X-ray photoelectron spectroscopy (XPS) analysis was performed by an XPS analyzer (Thermo Fisher Scientific, K-alpha, USA). UV-Vis Diffuse Reflectance Spectroscopy (DRS) spectroscopy was obtained in 200–800 nm region (UV-3600, Japan). The analysis of electron spin resonance (ESR) aimed to identify the active species using the trap reagent (DMPO) under the Xenon lamp (Bruker Corporation, Bruker A300, USA). The transient photocurrent responses and electrochemical impedance spectroscopy (EIS) were performed in a three-electrode cell with a CHI-760E workstation (USA).

**Photocatalytic experiments**

The photocatalytic activity was tested via the degradation of TC (20 mg·L$^{-1}$) in visible LED light (PCX-50B Multichannel photochemical reaction system, Beijing). 20 mg of samples were suspended in 40 mL TC solution. The suspension was magnetically stirred in the dark for 30 min to achieve the adsorption-desorption equilibrium. Afterwards, the suspension was irradiated in visible light for 180 min. 1.0 mL suspension was extracted at given time intervals and filtered with a filter membrane (0.22 µm). Then, the residual TC was analyzed by a microplate reader (Thermo Fisher Scientific, Varioskan LUX, USA) at 357 nm. The removal efficiency of TC and the first-order kinetics could be calculated by the following Eq. (1) (2):

\[
\eta = \frac{C_0 - C_t}{C_0} \times 100\% \\
\ln \left( \frac{C}{C_0} \right) = -kt \tag{2}
\]
where \( \eta \) was represented the removal efficiency of TC; \( C_0 \) was represented the initial concentration of TC; \( C_t \) was represented concentration of TC at irradiation time (t); \( k \) was the first-order rate constant.

**Toxicity evaluation**

The toxicity of TC and its intermediates was monitored using Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Bacillus subtilis) as the targets. In the test, the parent TC and intermediates at specific time intervals were mixed into the culture medium with bacteria separately. The bacterial suspensions were incubated at 37°C for 24 h at 160 rpm. The antimicrobial toxicity was estimated by measuring the absorbance of solutions at 600 nm, and calculated according to (3) (Coledam et al. 2016):

\[
\text{Inhibition index (I)} = \frac{A_0 - A}{A_0} \times 100\%
\]

where \( A_0 \) and \( A \) were the absorbance in the absence and presence of photocatalysts, respectively.

The acute toxicity of catalysts, TC solution, and treated TC solutions were assessed using zebrafish embryos (He et al. 2020). In each well of 96-well plates, one zebrafish embryo and 200 µL of suspension were added. H-buffer solution was used as the negative control. These culture plates were incubated at 28 ± 0.5 °C. The survival rate and hatchability of the zebrafish embryos were observed at 72 h. Each treatment was carried out in three replicates of 12 embryos each.

**Results And Discussion**

**Characterization of the synthesized photocatalysts**

**Structure and morphology characterization**

The XRD patterns were applied to analyze the crystal structure of the samples (Fig. 1a). All prepared samples had two typical diffraction peaks of g-C\(_3\)N\(_4\). The prominent diffraction peaks at \( \sim 12.8^\circ \) and \( \sim 27.5^\circ \) were associated with (100) and (002) planes of g-C\(_3\)N\(_4\), which belonged to the in-plane structural packing motif of tri-s-triazine units and interlayer stacking of aromatic segments, respectively (Chi et al. 2022, Dong et al. 2018). Once SA was introduced, the (002) peak intensities of CNU-SA-2, CNM-SA-0.5, and CND-SA-0.5 decreased due to the disturbance of graphitic structure. The changes in peak intensity confirmed that SA was introduced in the framework of g-C\(_3\)N\(_4\). Additionally, the (002) peak intensity of g-C\(_3\)N\(_4\) prepared with urea (CNU and CNU-SA-2) was weaker than those of g-C\(_3\)N\(_4\) prepared with melamine and dicyandiamide (CNM and CND, CNM-SA-0.5 and CND-SA-0.5), demonstrating relatively loose structure (Luo et al. 2020).
The functional groups of synthesized samples were analyzed by FT-IR spectroscopy (Fig. 1b). All samples maintained the same characteristic signals, indicating that the SA modification and different precursors did not change the functional groups of g-C$_3$N$_4$. The peaks from 1200–1700 cm$^{-1}$ belonged to the typical stretching modes of C-N heterocycles (Sun et al. 2021). The small peak at ~ 813 cm$^{-1}$ represented the characteristic breathing mode of s-triazine (Yu et al. 2021). The broad absorption bands at 3000–3400 cm$^{-1}$ were assigned to the vibration modes of the N-H and O-H stretches (Feng et al. 2016, Qin et al. 2021).

The surface composition of CNU-SA-2 was characterized by XPS spectra (Fig. S1). Three peaks of C 1s, N 1s and O 1s appeared in CNU-SA-2 (Fig. S1a). The C/N molar ratio in the CNU-SA-2 was 0.71, which was consistent with the theoretical value of g-C$_3$N$_4$ (0.75). As shown in the high-resolution C 1s spectra (Fig. S1b), two characteristic peaks at ~ 288.0 eV and ~ 284.5 eV, attributed to aromatic carbon atoms (N-C = N) and the sp$^2$ hybridized carbon (C-NH$_2$) respectively (Li et al. 2018). Three peaks at ~ 400.7 eV, ~ 399.6 eV, and ~ 398.6 eV in the high-resolution N 1s spectra were assigned to C-NH, N-C$_3$, and C-N = C groups, respectively (Fig. S1c). For O 1s spectrum (Fig. S1d), peaks at ~ 531.8 eV, ~ 531.9 eV, and ~ 532.5 eV belonging to -OH, C = O groups and N-C-O bonds respectively were observed (Jia et al. 2019, Zhou et al. 2019a).

The SEM images of CNU, CNM and CND exhibited similar stacked nanosheet morphologies with a porous surface (Fig. 2a, b, c). CNM and CND showed a tight aggregation of nanoplates. CNU had loose sheets with reduced thickness due to the gaseous CO$_2$ and H$_2$O molecules generated during synthesis. The reduction in thickness of CNU might benefit the migration of charge carriers and introduce more active sites. After SA addition, the nanosheets of CNU-SA-2 tended to curve, and CNM-SA-0.5 and CND-SA-0.5 presented similar morphologies as CNM and CND (Fig. 2d, e, f).

The specific surface area and porous structure of synthesized photocatalysts were verified by N$_2$ adsorption-desorption isotherm (Fig. S2). All samples presented type IV isotherms with H1 hysteresis loops, indicating the formation of mesoporous structure. Compared to CNM (26.89 m$^2$/g) and CND (20.43 m$^2$/g), CNU possessed a higher surface area (90.11 m$^2$/g) (Table S1). After SA addition, the specific surface area of CNU-SA-2 decreased while the pore volume reduced from 0.56 to 0.19 cm$^3$/g since the addition of SA destructed the intrinsic structural units and collapsed the pores. The specific surface area of CNM-SA-0.5 and CND-SA-0.5 had no obvious change, which illustrated that the introduction of SA did not cause lattice collapse in g-C$_3$N$_4$ prepared with melamine and dicyandiamide.

**Optical and electrochemical properties**

The optical properties were assessed by UV-Vis DRS. All samples exhibited visible-light absorption. The adsorption edges of pure g-C$_3$N$_4$ were > 450 nm (Fig. 3a). After the addition of SA, CNU-SA-2, CNM-SA-0.5 and CND-SA-0.5 showed a red shift, confirming the momentous effect due to the incorporation of SA into the g-C$_3$N$_4$ framework. Using Kubelka-Munk transformation ($\alpha$hv = $A$(hv-Eg)$^{n/2}$) (Shi et al. 2020), the band gap (Eg) values of CNU, CNM, CND, CNU-SA-2, CNM-SA-0.5, and CND-SA-0.5 were 2.60, 2.46, 2.52, 2.21,
2.34, and 2.42 eV, respectively (Fig. 3b). In comparison to pure g-C$_3$N$_4$, the band gaps of CNU-SA-2, CNM-SA-0.5, and CND-SA-0.5 were narrower, enhancing the visible light harvesting. Among the modified g-C$_3$N$_4$ samples, CNU-SA-2 had the greatest light response in the visible light region.

To confirm efficient photogenerated charge separation and migration after the addition of SA in CNU, the photocurrent response was investigated. Figure 3c showed the transient photocurrent measurements of CNU and CNU-SA-2 via several on-off cycles under visible Xenon lamp irradiation (200 W). CNU-SA-2 displayed higher photocurrent response than CNU, implying reduced photo-induced charge carrier recombination due to the introduction of SA. The smaller impedance arc radius of CNU-SA-2 than that of CNU (Fig. 3d, measured by EIS) suggested that the addition of SA promoted photogenerated charge transfer efficiency.

### Photocatalytic performance of synthesized samples

The photocatalytic performance was studied by degrading TC under visible LED light (Fig. 4). The adsorption-desorption equilibrium could be reached in 30 min in the dark. The adsorption efficiency of all samples was less 14.7%. The CNU and CNU-SA-x products had higher adsorption capacity than the CNM, CNM-SA-x, CND and CND-SA-x, due to the relatively higher specific surface area (Table S1).

TC was hardly degraded without catalysts under visible light irradiation. All SA-modified products exhibited superior photocatalytic performance than the corresponding pristine g-C$_3$N$_4$ (Fig. 4). After the addition of SA, the highest reaction rate rates ($k$) were observed (Fig. 4 (inserts)), suggesting that optimal doping amounts of SA could enhance the photocatalytic efficiency. Among these photocatalysts, the CNU-SA-2 displayed the best photocatalytic activity of 82.3% within 180 min under the visible LED light. The $k$ value of CNU-SA-2 (0.0095 min$^{-1}$) was about twice high as that of CNU (0.0046 min$^{-1}$). Hence, CNU-SA-2 was chosen for all subsequent tests.

### Practicability of CNU-SA-2

#### Effect of the initial pH

The initial pH is an important parameter in the photocatalysis. The effect of the initial pH on photocatalytic activity was assessed in the pH range of 3–10 (Fig. 5a). The removal efficiency of TC increased from 74.0–82.3% with the increase of pH from 3.08 to 5.53, then decreased to 80.6% and 77.8% with further increase of pH to 8.12 and 10.04. The TC degradation efficiency peaked at 82.3% with an initial pH of 5.53. TC molecules exist as TCH$^+$ cationic species (pH < 3.3), zwitterionic species (pH = 3.3–7.7), TCH$^-$ anionic species (7.7 < pH < 9.0), and TC$^{2-}$ anionic species (pH > 9.0) (Chao et al. 2017, Ye et al. 2019). Since the pH$_{pzc}$ of CNU-SA-2 was ~ 5.73 (Text S2 and Fig. S3), the electrostatic interaction between CNU-SA-2 and TC was repulsive at pH 3.08, 8.12 and 10.04. The electrostatic repulsion between CNU-SA-2 and TC was lowest at pH 5.53, resulting in the highest adsorption capacity promoting further TC degradation (Li et al. 2022). On the other hand, photodegradation of TC was favored under the weakly alkaline condition (pH 8.12 and pH 10.04) since the deprotonated TC species are instable (Zhang et al.
Overall, the photocatalytic degradation performance of TC by CNU-SA-2 remained above 74.0% over a wide pH range, revealing potential practical application in real-life water bodies.

**Effect of inorganic anions and natural organic matter**

As an integral part of real-life water bodies, the inorganic anions (Cl\(^{-}\), CO\(_3\)^{2-}, H\(_2\)PO\(_4\)^{-}, and SO\(_4\)^{2-}) and natural organic matters (humic acid (HA)) may affect the degradation performance of photocatalysts (Du et al. 2021, Zhao et al. 2021). Therefore, it is necessary to explore their influences on the photocatalytic activity (Fig. 5b). The degradation efficiency of TC fell from 82.3–54.1% in the presence of HA (10 mg/L), which might be due to the light attenuation and radicals scavenger of HA, and competition of adsorption active sites between HA and TC (Zhao et al. 2021).

By contrast, the removal efficiency of TC by CNU-SA-2 in the presence of different anions declined at acceptable levels. The H\(_2\)PO\(_4\)^{-} and SO\(_4\)^{2-} hindered the adsorption capacity of CNU-SA-2 due to competition between the anions and TC, while other anions had no significant effect on the adsorption process. The H\(_2\)PO\(_4\)^{-} and SO\(_4\)^{2-} anions could react with h\(^{+}\) and •OH, thus leading to the inhibition of TC degradation. The coexistence of Cl\(^{-}\) had little negative effect on the degradation of TC due to generation of Cl• with weaker oxidative ability (Tang et al. 2022). The CO\(_3\)^{2-} anion could consume h\(^{+}\) and •OH to induce CO\(_3\)^{-} with less reactivity to affect TC degradation (Liu et al. 2022).

**Degradation of four different TCs**

To testify the universal effectiveness, the removals of various TCs (tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC), and doxycycline (DXC)) were conducted (Fig. 5c). CNU-SA-2 could absorb TC (14.7%) and DTC (7.6%) at an initial concentration of 20 mg/L, while no obvious adsorption towards CTC and OTC. The subsequent 180-min photocatalysis under visible light increased the removal efficiency of TC, DTC, CTC, and OTC to 82.3%, 55.3%, 72.1%, and 46.9%, respectively. These results demonstrated that the CNU-SA-2 photocatalyst could damage the tetraphenyl backbone of the TCs.

**Recyclability**

The reusability of photocatalysts also plays significant roles in the practical applications. The performance of four repeated cycle tests of CNU-SA-2 on TC degradation was studied. After each cycle, the used CNU-SA-2 was separated by centrifugation, washed thoroughly with water, and dried at 80 °C. As shown in Fig. 5d, CNU-SA-2 exhibited a good stability and maintained the photocatalytic efficiency of ~ 80% after four cycles. Hence, the results indicated CNU-SA-2 catalyst possessed excellent reusability in the photocatalytic application.

**Photocatalytic mechanism**

Above results suggested that the incorporation of benzene-ring to g-C\(_3\)N\(_4\) could prompt the photocatalytic efficiency. To further verify the conjugated effect of the numbers of benzene rings on the photocatalytic performance of CNU, CNU-Naphthoic acid was synthesized by thermal polymerization of urea and
naphthoic acid with two benzene rings. The amount of naphthoic acid was estimated based on the portion of benzene rings (56.5 wt%) in SA. As shown in Fig. 6a, CN-SA-2 showed the highest photocatalytic activity for TC degradation compared to CNU and CNU-Naphthoic acid. The TC degradation efficiency in the presence of CNU-Naphthoic acid was 67.4% in 180 min, i.e., an 9.6% enhancement relative to pristine CNU. Thus, the single/double benzene rings-modified CNU exhibited higher photocatalytic efficiency than that of CNU due to the accelerated mobility of electron-hole pairs in the modified g-C$_3$N$_4$ photocatalysts. But the photocatalytic performance of CNU-Naphthoic acid was inferior to that of CNU-SA-2. The double benzene rings doping in the CNU network might result in weaker separation of photogenerated charge carriers (Li et al. 2018). Therefore, a single benzene ring was crucial to improve the photocatalytic performance for TC degradation.

To investigate the mechanism of CNU-SA-2 on the TC degradation under visible light, the generated active species were determined using the free radical trapping tests. Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na, 1 mM), isopropanol (IPA, 1 mM), and benzoquinone (BQ, 1 mM) acted as the quenchers of holes (h$^+$), hydroxyl radical (•OH), and superoxide radical (•O$_2^-$) respectively. As shown in Fig. 6a, the addition of IPA had a minor effect on the photocatalytic efficiency, while the addition of EDTA-2Na and BQ had significant effects. The results indicated that h$^+$ and •O$_2^-$ played important roles for the degradation of TC. ESR technique was further employed to detect the reactive species (•OH and •O$_2^-$) in the photocatalytic system (Fig. 6b, c). DMPO-•OH and DMPO-•O$_2^-$ were not detected in the dark but became detectable under visible light irradiation for 10 min, confirming that CNU-SA-2 could form free radicals under light irradiation, same as the above trapping experiments.

Based on above analyses, the mechanism of TC removal by CNU-SA-2 was proposed in Scheme 1. Under visible light irradiation, CNU-SA-2 with a single benzene ring could be excited, where electrons (e$^-$) were produced in the conduction band and h$^+$ in the valence band. The e$^-$ in the conduction band trapped O$_2$ to produce •O$_2^-$, which further served as one of the major active species for TC degradation. The h$^+$ in the valence band could directly oxidize TC. •OH could be produced via the reaction between •O$_2^-$, H$^+$, and e$^-$, and served as an active species in the degradation. Thus, h$^+$, •OH, and •O$_2^-$ could synergistically degrade TC under visible light.

**Toxicity assessment**

Considering the real-life application of the synthesized CNU-SA-2 photocatalyst, it was necessary to assess the potential environmental impact of TC and its degradation products. Firstly, antimicrobial tests based on *E. coli* and *B. subtilis* were performed. As shown in Fig. 7a, the *E. coli* and *B. subtilis* inhibition ratio gradually fell to 32.2% and 25.6%, respectively after 180 min of TC degradation. The decrease in the bacterial inhibition ratio indicated that most intermediates had reduced antibacterial activity.

Toxicity assessment is important in some cases, which is due to the toxicity of some degradation TC products may be higher than that of the parent TC. The zebrafish embryo is an attractive biological model...
due to its fecundity, optical transparency and genetic malleability (Sharma & Saneja 2022). Furthermore, the acute toxicity of TC and the degraded intermediates on zebrafish embryos was also examined. The hatchability and survival rate of zebrafish embryos in the CNU-SA-2 photocatalytic system was investigated (Fig. 7b). TC solution without any treatment showed 100% hatchability and survival rate of the embryos after an incubation of 72 h. There was no significant change in hatchability and survival rate of the zebrafish embryos with increasing duration of the CNU-SA-2 photocatalytic reaction (Fig. 7b). It was reasonable to conclude that the TC intermediates had no toxic impact on the zebrafish embryos.

The biosafety of CNU-SA-2 photocatalyst was also evaluated using zebrafish embryos. Zebrafish embryos were treated with CNU-SA-2 at different dosages (0.13, 0.25, 0.50, 0.75, and 1.0 g/L) for 72 h. The hatching and survival rates of zebrafish embryos were above 80% during the exposure period (Fig. 7c), indicating no significant toxicity of CNU-SA-2 on zebrafish embryos.

**Conclusion**

In summary, a series of SA-modified g-C$_3$N$_4$ samples were synthesized via copolymerization using different precursors (urea, melamine and dicyandiamide). The doping of SA with a benzene ring in g-C$_3$N$_4$ framework showed extended visible-light absorbance and decreased recombination of electron-hole pairs, leading to effective TC removal under visible light. The CNU-SA-2 achieved a photocatalytic rate of 0.0095 min$^{-1}$ for TC degradation, which was 2 times higher than that of CNU. Various experimental conditions (initial pH values, co-existing anions, HA and different TCs) were evaluated to demonstrate the potential of the photocatalyst for environmental remediation. Additionally, the photocatalytic activity of CNU-SA-2 remained stable after four cycle tests. The trapping tests and ESR analyses revealed that h$^+$ and ·O$_2$$^-$ were the main active species in the photocatalytic process of TC degradation. The bacterial inhibition tests and hatching/survival tests of zebrafish embryos revealed the reduced toxicity of the intermediates and no acute toxicity of CNU-SA-2. This study offers a scheme for improving photocatalytic activity of g-C$_3$N$_4$ for remediation of real-life antibiotics-contaminated water.

**Declarations**

**Author contribution**

Mengmeng Chen: Conceptualization, Data curation, Investigation, Methodology, Writing - review & editing. Mengxue Li: Writing - review & editing. Peng Li: Writing - review & editing. Stephanie Ling Jie Lee: Writing - review & editing. Juanjuan Tang: Data curation. Qian Li: Data curation. Sijie Lin: Project design, Supervision, Writing - review & editing, Project administration, Funding acquisition.

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Data availability

All data generated or analyzed during the current work are included in this published article and its supplementary information files.

Ethics approval

All animal tests were in accordance with the current Chinese legislation and the Animal Ethics Committee at Tongji University.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Chao Y, Yang L, Ji H, Zhu W, Pang J, Han C, Li H (2017) Graphene-analogue molybdenum disulfide for adsorptive removal of tetracycline from aqueous solution: equilibrium, kinetic, and thermodynamic studies. Environ Prog Sustain 36:815–821

2. Chi X, Liu F, Gao Y, Song J, Guan R, Yuan H (2022) An efficient B/Na co-doped porous g-C3N4 nanosheets photocatalyst with enhanced photocatalytic hydrogen evolution and degradation of tetracycline under visible light. Appl Surf Sci 576:151837

3. Coledam DA, Aquino JM, Silva BF, Silva AJ, Rocha-Filho RC (2016) Electrochemical mineralization of norfloxacin using distinct boron-doped diamond anodes in a filter-press reactor, with investigations of toxicity and oxidation by-products. Electrochim Acta 213:856–864

4. Dong H, Guo X, Yang C, Ouyang Z (2018) Synthesis of g-C3N4 by different precursors under burning explosion effect and its photocatalytic degradation for tylosin. Appl Catal B- Environ 230:65–76

5. Du J, Xu Z, Li H, Yang H, Xu S, Wang J, Jia Y, Ma S, Zhan S (2021) Ag3PO4/g-C3N4 Z-scheme composites with enhanced visible-light-driven disinfection and organic pollutants degradation: Uncovering the mechanism. Appl Surf Sci 541:148487

6. Feng J, Chen T, Liu S, Zhou Q, Ren Y, Lv Y, Fan Z (2016) Improvement of g-C3N4 photocatalytic properties using the Hummers method. J Colloid Interface Sci 479:1–6

7. Han C-H, Park H-D, Kim S-B, Yargeau V, Choi J-W, Lee S-H, Park J-A (2020) Oxidation of tetracycline and oxytetracycline for the photo-Fenton process: Their transformation products and toxicity assessment. Water Res 172:115514

8. He Y, Peng G, Jiang Y, Zhao M, Wang X, Chen M, Lin S (2020) Environmental Hazard Potential of Nano-Photocatalysts Determined by Nano-Bio Interactions and Exposure Conditions. Small 16:e1907690

9. Jia L, Cheng X, Wang X, Cai H, He P, Ma J, Li L, Ding Y, Fan X (2019) Large-Scale Preparation of g-C3N4 Porous Nanotubes with Enhanced Photocatalytic Activity by Using Salicylic Acid and
Melamine. Ind Eng Chem Res 59:1065–1072

10. Li M, Li P, Zhang L, Chen M, Tang J, Qin C, Ling Jie Lee S, Lin S (2022) Facile fabrication of ZnO decorated ZnFe-layered double hydroxides @ biochar nanocomposites for synergistic photodegradation of tetracycline under visible light. Chem Eng J 434:134772

11. Li Z, Chen H, Liu W (2018) Full-Spectrum Photocatalytic Activity of ZnO/CuO/ZnFe2O4 Nanocomposite as a PhotoFenton-Like Catalyst. Catalysts 8:557

12. Lin B, Li S, Peng Y, Chen Z, Wang X (2021) MOF-derived core/shell C-TiO2/CoTiO3 type II heterojunction for efficient photocatalytic removal of antibiotics. J Hazard Mater 406:124675

13. Lin S, Yu T, Yu Z, Hu X, Yin D (2018) Nanomaterials safer-by-design: an environmental safety perspective. Adv Mater 30:1705691

14. Liu N, Lu N, Su Y, Wang P, Quan X (2019a) Fabrication of g-C3N4/Ti3C2 composite and its visible-light photocatalytic capability for ciprofloxacin degradation. Sep Purif Technol 211:782–789

15. Liu S, Wang S, Jiang Y, Zhao Z, Jiang G, Sun Z (2019b) Synthesis of Fe2O3 loaded porous g-C3N4 photocatalyst for photocatalytic reduction of dinitrogen to ammonia. Chem Eng J 373:572–579

16. Liu Y, Wang X, Sun Q, Yuan M, Sun Z, Xia S, Zhao J (2022) Enhanced visible light photo-Fenton-like degradation of tetracyclines by expanded perlite supported FeMo3Ox/g-C3N4 floating Z-scheme catalyst. J Hazard Mater 424:127387

17. Luo Z, Song Y, Wang M, Zheng X, Qu L, Wang J, Wu X, Wu Z (2020) Comparison of g-C3N4 synthesized by different precursors in remediation of phenanthrene contaminated soil and ecotoxicity. J Photoch Photobio A 389:112241

18. Osin OA, Yu T, Cai X, Jiang Y, Peng G, Cheng X, Li R, Qin Y, Lin S (2018) Photocatalytic Degradation of 4-Nitrophenol by C, N-TiO2: Degradation Efficiency vs. Embryonic Toxicity of the Resulting Compounds. Front Chem 6:192

19. Qin Y, Lu J, Zhao X, Lin X, Hao Y, Huo P, Meng M, Yan Y (2021) Nitrogen defect engineering and π-conjugation structure decorated g-C3N4 with highly enhanced visible-light photocatalytic hydrogen evolution and mechanism insight. Chem Eng J 425:131844

20. Sharma A, Saneja A (2022) Zebrafish as a powerful alternative model organism for preclinical investigation of nanomedicines. Drug Discov Today 27:1513–1522

21. Shi Y, Li J, Wan D, Huang J, Liu Y (2020) Peroxymonosulfate-enhanced photocatalysis by carbonyl-modified g-C3N4 for effective degradation of the tetracycline hydrochloride. Sci Total Environ 749:142313

22. Sun H, Guo F, Pan J, Huang W, Wang K, Shi W (2021) One-pot thermal polymerization route to prepare N-deficient modified g-C3N4 for the degradation of tetracycline by the synergistic effect of photocatalysis and persulfate-based advanced oxidation process. Chem Eng J 406:126844

23. Tang J, Wang J, Tang L, Feng C, Zhu X, Yi Y, Feng H, Yu J, Ren X (2022) Preparation of floating porous g-C3N4 photocatalyst via a facile one-pot method for efficient photocatalytic elimination of tetracycline under visible light irradiation. Chem Eng J 430:132669
24. Watkinson A, Murby E, Costanzo S (2007) Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. Water Res 41:4164–4176

25. Wei R, Ge F, Chen M, Huang S, Wang R (2010) Pollution of tetracyclines from livestock and poultry farms in aquatic environment in Jiangsu Province, China. J Agro-Environment Sci 29:1205

26. Xia P, Zhu B, Yu J, Cao S, Jaroniec M (2017) Ultra-thin nanosheet assemblies of graphitic carbon nitride for enhanced photocatalytic CO2 reduction. J Mater Chem A 5:3230–3238

27. Xu L, Zhang H, Xiong P, Zhu Q, Liao C, Jiang G (2021) Occurrence, fate, and risk assessment of typical tetracycline antibiotics in the aquatic environment: A review. Sci Total Environ 753:141975

28. Ye S, Yan M, Tan X, Liang J, Zeng G, Wu H, Song B, Zhou C, Yang Y, Wang H (2019) Facile assembled biochar-based nanocomposite with improved graphitization for efficient photocatalytic activity driven by visible light. Appl Catal B-Environ 250:78–88

29. Yu Y, Wu K, Xu W, Chen D, Fang J, Zhu X, Sun J, Liang Y, Hu X, Li R (2021) Adsorption-photocatalysis synergistic removal of contaminants under antibiotic and Cr (VI) coexistence environment using non-metal g-C3N4 based nanomaterial obtained by supramolecular self-assembly method. J Hazard Mater 404:124171

30. Zhang J, Zhang G, Chen X, Lin S, Mohlmann L, Dolega G, Lipner G, Antonietti M, Blechert S, Wang X (2012) Co-monomer control of carbon nitride semiconductors to optimize hydrogen evolution with visible light. Angew Chem Int Ed Engl 51:3183–3187

31. Zhang Q, Jiang L, Wang J, Zhu Y, Pu Y, Dai W (2020) Photocatalytic degradation of tetracycline antibiotics using three-dimensional network structure perylene diimide supramolecular organic photocatalyst under visible-light irradiation. Appl Catal B-Environ 277:119122

32. Zhao G, Ding J, Zhou F, Chen X, Wei L, Gao Q, Wang K, Zhao Q (2021) Construction of a visible-light-driven magnetic dual Z-scheme BiVO4/g-C3N4/NiFe2O4 photocatalyst for effective removal of ofloxacin: Mechanisms and degradation pathway. Chem Eng J 405:126704

33. Zhao T, Zheng M, Fu C, Li G, Xiong Y, Qiu W, Zhang T, Zhang J, Zheng C (2020) Effect of low-level H2O2 and Fe (II) on the UV treatment of tetracycline antibiotics and the toxicity of reaction solutions to zebrafish embryos. Chem Eng J 394:125021

34. Zhou C, Huang D, Xu P, Zeng G, Huang J, Shi T, Lai C, Zhang C, Cheng M, Lu Y, Duan A, Xiong W, Zhou M (2019a) Efficient visible light driven degradation of sulfamethazine and tetracycline by salicylic acid modified polymeric carbon nitride via charge transfer. Chem Eng J 370:1077–1086

35. Zhou C, Zeng Z, Zeng G, Huang D, Xiao R, Cheng M, Zhang C, Xiong W, Lai C, Yang Y, Wang W, Yi H, Li B (2019b) Visible-light-driven photocatalytic degradation of sulfamethazine by surface engineering of carbon nitrideProperties, degradation pathway and mechanisms. J Hazard Mater 380:120815

36. Zhu A, Qiao L, Tan P, Pan J (2020) Interfaces of graphitic carbon nitride-based composite photocatalysts. Inorg Chem Front 7:4754–4793
Scheme 1 is available in the Supplementary Files section

**Figures**

Figure 1

XRD patterns (a) and FT-IR spectra (b) of synthesized g-C$_3$N$_4$ photocatalysts.

Figure 2

SEM images of CNU (a), CNM (b), CND (c), CNU-SA-2 (d), CNM-SA-0.5 (e) and CND-SA-0.5 (f).
Figure 3

UV-vis diffuse reflectance spectra (a) and the corresponding band gap energy (b) of synthesized samples; transient photocurrent responses (c) and EIS Nyquist plots (d) of CNU and CNU-SA-2.

Figure 4

Photocatalytic degradation of TC by the various synthesized g-C₃N₄ photocatalysts. The inserts were the corresponding rate constants.

Figure 5
Effects of initial pH (a), inorganic anions and HA (b) on CNU-SA-2 for TC degradation; photocatalytic degradation efficiency for four different TCs by CNU-SA-2 (c); the cycle tests of CNU-SA-0.2 for degradation TC (d).

Figure 6

Comparison of TC removal efficiencies by CNU, CNU-SA-2 and CNU-Naphthoic acid (a); scavengers trapping studies in TC degradation by CNU-SA-2 under visible light irradiation (b); the ESR spectra of free radicals trapped by DMPO (·OH and ·O$_2^-$) in CNU-SA-2 dispersion in dark and under visible light irradiation (c) (d).

Figure 7

Antibacterial activity of effluent during the photocatalytic degradation of TC by CNU-SA-2 (a); hatching rate and survival rate of zebrafish embryos exposed to TC and its intermediates in CNU-SA-2 photocatalytic system (b), and at different dosages of CNU-SA-2 (c).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.png
- Supportinginformation.docx