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Tannic Acid-Based Organic–Inorganic Nanoflowers with Peroxidase-Like Activity for Dye Degradation

Abstract

Organic–inorganic hybrid nanoflowers (NFs) have emerged as promising nanozymes owing to their remarkable enzyme-like catalytic properties. Accordingly, this study aimed to synthesize tannic acid NFs (TA NFs) and investigate their peroxidase-like activity and their dye degradation performance. The synthesis procedure are based on self-assembly process involving TA as organic components and Cu^{2+} ions as inorganic components. The peroxidase-like catalytic activity of the synthesized TA NFs was investigated by monitoring the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 via a Fenton-like reaction. Furthermore, dye degradation performance of TA NFs was evaluated using acid orange 7 dye as a model dye. Under the optimized conditions (pH 10 and 1 mM H_2O_2), the TA NFs achieved 94% decolorization of AO 7 within 15 min through the oxidative degradation of the dye by hydroxyl radicals (OH•) generated during the Fenton-like reaction. These findings suggest that TA NFs represent a promising nanozyme platform for environmental remediation, particularly for the rapid degradation of azo dyes in wastewater treatment. Owing to their facile synthesis, high catalytic activity, and excellent degradation efficiency, TA NFs have considerable potential as cost-effective and sustainable catalysts for removal of organic dye pollutants.

Keywords: Acid orange 7, Catalytic activity, Dye degradation, Organic inorganic hybrid nanoflowers, Tannic acid

Boya Giderimi İçin Peroksidaz Benzeri Aktiviteye Sahip Tannik Asit Tabanlı Organik–İnorganik Nanoçiçekler

Öz

Organik inorganik hibrit nanoçiçekler (NÇ) dikkat çekici enzim benzeri katalitik özellikleri sayesinde gelecek vaat eden nanoenzimler olarak öne çıkmıştır. Bu bağlamda, bu çalışmanın amacı, tannik asit NÇ'leri (TA NÇ) sentezlemek ve bunların peroksidaz benzeri aktivitesini ve boya bozunma performansını incelemektir. Sentez prosedürü, organik bileşenler olarak TA'yı ve inorganik bileşenler olarak Cu^{+2} iyonlarını içeren kendiliğinden birleşme sürecine dayanmaktadır. Sentezlenen TA NÇ'lerin peroksidaz benzeri aktiviteleri Fenton benzeri bir reaksiyon aracılığıyla H_2O_2 varlığında 3,3′,5,5′-tetrametilbenzidin (TMB) maddesinin oksidasyonunun izlenmesi suretiyle incelenmiştir. Ayrıca TA NÇ'lerin boya giderim performansları model boya olarak seçilen asit turuncu 7 (AT 7) kullanılarak tespit edilmiştir. Optimize edilmiş koşullar altında (pH 10 ve 1 mM H_2O_2), TA NÇ'leri Fenton benzeri reaksiyon sırasında üretilen hidroksil radikalleri (OH•) aracılığıyla boyanın oksidatif bozunması yoluyla 15 dakika içinde AT 7'nin %94 oranında renk giderimini sağlamıştır. Bu bulgular, TA NÇ'lerin çevresel iyileştirme, özellikle de atık su arıtımında azo boyalarının hızla parçalanması için umut verici bir

nanozim platformu oluşturduğunu göstermektedir. Kolay sentezlenebilirlikleri, yüksek katalitik aktiviteleri ve mükemmel bozunma verimlilikleri sayesinde TA NÇ'ler, organik boya kirleticilerinin giderilmesinde uygun maliyetli ve sürdürülebilir katalizörler olarak kayda değer bir potansiyele sahiptir.

Anahtar kelimeler: Asit turuncu 7, Katalitik aktivite, Boya giderimi, Organik inorganik hibrit nanoçiçekler, Tannik asit

1.INTRODUCTION

Organic inorganic hybrid nanoflowers (NFs) are a distinctive class of nanostructures characterized by their flower-like morphology, which provides a high surface-to-volume ratio and abundant active sites, leading to enhanced catalytic activity [1,2]. These hierarchical architectures are typically synthesized through controlled self-assembly processes involving organic components such as enzyme, protein and biomolecules and inorganic components including Cu^{2+}, Co^{2+}, Mg^{2+} and Zn^{2+} ions [3]. The unique structural features of NFs, such as their porous and multilayered arrangement, facilitate enhanced mass transfer which result in more interaction of the substrate to the active site of enzymes, leading to increasing of catalytic activity of enzymes [4]. The integration of organic molecules with inorganic materials in these NFs imparts synergistic properties, including improved mechanical strength, reusability and recovery from the reaction systems, which are essential for practical uses in different application areas [5].

In recent years, enzyme mimicking NFs have attracted significant attention due to their ability to replicate natural enzyme functions as an alternative to enzymes [6]. These NFs can be synthesized with the non protein molecules by the controlled self-assembly techniques that promote the formation hierarchical, flower-like morphology [7]. These NFs possess enzyme-like catalytic properties similar to natural enzymes, including peroxidase [8], oxidase [9], and laccase [10], enabling them to catalyze specific chemical reactions. The catalytic mechanisms of these enzyme-mimicking NFs arise from their ability to facilitate redox reactions similar to natural enzymes. For instance, peroxidase-mimicking NFs catalyze the decomposition of hydrogen peroxide (H_2O_2) to generate reactive radicals that oxidize substrat molecules, leading to their oxidation. Additionally, the porous structure ensures effective mass transport of reactants and products, maintaining high catalytic turnover rates under mild environmental conditions [8]. The enhanced catalytic activity and outstanding stability of NFs have enabled their extensive use in various fields, including biosensors [11], bioremediation [12], biomimetic catalyst [10] and dye degradation [7]. This enzyme-like behavior is particularly valuable for environmental remediation, where these NFs can catalyze the degradation of hazardous dyes in wastewater. Their catalytic efficiency, combined with robustness against harsh conditions and recyclability, makes them as promising candidates for sustainable and cost-effective dye removal technologies [13].

Fenton and Fenton like systems are an important area of research that has been studied for over 100 years and has attracted significant interest due to their broad potential in various fields of application [33]. In these systems, transition metals (Fe, Cu, Mn, Ag, Co, etc.) or nanozymes catalyze H_2O_2, leading to the formation of highly reactive oxidizing species like hydroxyl and hydroperoxyl radicals [34]. Due to its strong oxidative capacity, the Fenton reactions have been widely employed to degrade organic pollutants such as organic dyes, pesticides, and organic solvents etc. [35]. The generated radicals can interact with organic pollutants through various oxidative pathways, including electron transfer, radical addition and hydrogen atom abstraction. These reactions can disrupt the chemical structure of organic molecules, leading to their transformation into harmless compounds like CO_2, H_2O and inorganic salts [36].

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Synthetic dyes are extensively used in many industrial process including plastic, paper, food and textile. They have toxic effects to water and soil [14]. Azo dyes are the most abundant class of synthetic dyes that are soluble in water. Azo dyes contain one or more azo bonds (-N-N-) typically attached to complex aromatic structures [15]. Acid orange 7 (AO 7) is a widely used water soluble anionic azo dye containing aromatic azo bond and naphthalene ring in textile and paper industry [16]. However the extensive use of AO 7 can lead to generation of toxic aromatic compounds [17]. Therefore, the efficient removal of AO 7 from wastewater has become an essential process, particularly through advanced oxidation, adsorption and catalytic degradation methods [18, 19]. Among these approaches, Fenton/Fenton-like reactions have been used for the degradation of AO 7 due to their ability to generate highly reactive oxygen species through H_2O_2 activation. Several studies have demonstrated the effective degradation of AO 7 using Fenton-like systems [37-39].

Herein, Tannic acid NFs (TA NFs) were synthesized by employing tannic acid (TA) as the organic building block and copper(II) ions as the inorganic component. TA was selected as the organic component because of its abundant phenolic hydroxyl groups, which can strongly coordinate with Cu^{2+} ions and facilitate the formation of stable NFs structures. In addition, the polyphenolic structure of TA can participate in electron transfer processes and may contribute to the catalytic activation of H_2O_2. Owing to the reducing capability of TA, Cu^{2+} can be reduced to Cu^{+}, which promotes the generation of highly reactive hydroxyl radicals through a Fenton-like reaction [40]. Thus, TA not only serves as a structural component for the formation of the NFs but may also contribute to their catalytic activity by facilitating Cu^{2+}/Cu^{+} redox cycling and H_2O_2 activation. Accordingly, the catalytic activity and dye degradation performance of TA NFs were investigated through a Fenton-like reaction. AO 7 was selected as a model dye to evaluate the degradation performance of TA NFs for several reasons. Firstly, AO 7 is mono azo dye containing aromatic azo bond and naphthalene ring, making it a suitable pollutant for testing oxidation process. Secondly, its sulfonate groups on its structure provides excellent water solubility [16] and finally, AO 7 exhibits a stable absorbance peak at 485 nm, which enables accurate spectrophotometric monitoring of breakdown of chemical structure during catalytic degradation.

The results suggest that TA NFs constitue an efficient nanozyme platform for environmental remediation, particularly for the rapid degradation of azo dyes in wastewater treatment.

2. MATERIALS AND METHODS

2.1. Materials

Tannic acid (TA) (ACS reagent), phosphate buffered saline (PBS) tablets, copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$) (ACS reagent, ≥98.0%) and acid orange 7 (AO 7), acetic acid (glacial, ACS reagent, ≥99.7%), 3,3′,5,5′-Tetramethylbenzidine (TMB) (≥98%) and sodium carbonate (≥99.5%) were purchased from Sigma-Aldrich Co. Ltd. Sodium acetate anhydrous was purchased from Fisher Scientific Co. Ltd. Hydrogen peroxide (H_2O_2) (35%) was purchased from Merck Millipore Co. Ltd.

UV–Vis spectrophotometry (Shimadzu UV1800) was used for determination of peroxidase like activity and dye degradation performance of NFs. Scanning Electron Microscopy (SEM, ZEISS Gemini 500) was used for imaging of the morphologies of NFs.

2.2. Synthesis of Tannic Acid Nanoflowers

Tannic acid and Cu^{2+} ions were used as the organic and inorganic components of TA NFs, respectively. Firstly, $CuSO_4.5H_2O$ (120 mM) and TA (1 mg ml^{-1}) stock solutions were prepared by using deionized water. An aqueous solution of TA was added to phosphate buffer saline (pH 7.4, 10 mM). The final concentration of TA was 0.02 mg ml^{-1}. Afterwards, $CuSO_4$ (660 µL) was added to mixture and vigorously stirred at room temperature (RT) for 15 minutes. The mixture was left to incubation for 3

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days at RT. After the incubation, the precipitate was collected by using centrifuge (5,000 rpm, 10 min). The precipitate was dried in the oven at 40 °C [8].

2.3. Catalytic Activities of Tannic Acid Nanoflowers

The peroxidase like activities of TA NFs were determined by the oxidation of 3,3',5,5' - Tetramethylbenzidine (TMB). TMB (12 µL, 10 mM) was added to the mixture containing TA NFs (240 µL, 5 mg ml^{-1}), different concentration of H_2O_2 in sodium acetate buffer (10 mM, pH 4.0). The oxidation of TMB to the blue colored TMB_{ox} was monitored in UV-Vis Spectrometer.

A schematic representation of the proposed Fenton-like catalytic mechanism of TA NFs is presented in Equation 1 [23].

Equation 1:

$$Cu^{2+} + H_2O_2 \longrightarrow Cu^{+} + HOO\cdot + H^{+}$$

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + HO\cdot + OH^{-}$$

2.4. Dye Degradation Performance of Tannic Acid Nanoflowers:

The catalytic degradation of Acid Orange 7 (AO 7) by TA NFs was evaluated. 1 mg ml^{-1} TA NFs was mixed with 10 mg L^{-1}, 30 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1} AO 7 solutions containing 1 mM H_2O_2 in carbonate buffer (pH 10, 10 mM). The reaction mixture was incubated at RT for 15 min. The TA NFs were separated by centrifugation (10,000 rpm, 3 min). The absorbance values of supernatant was measured at 485 nm. The percentage of dye degradation was calculated using the Equation 1 [20].

% Dye Degradation=[(A0-At)/A0]*100

A_0= The absorbance values at 0 min.

A_t= The absorbance values at a certain time.

The dye degradation performance of TA NFs was investigated in different H_2O_2 concentrations (0 mM, 0.2 mM, 0.5 mM, 1 mM) and different buffers (acetate buffer pH4, PBS buffer pH7, carbonate bicarbonate buffer pH10).

3. RESULTS AND DISCUSSION

3.1. The Characterization of Tannic Acid Nanoflowers

The synthesis of NFs are based on the coordination reaction between hydroxyl and carboxylate groups in TA with Cu^{2+} ions in $Cu_3(PO_4)_2$ crystals. In the mechanism; initially, Cu^{2+} ions generated $Cu_3(PO_4)_2$ crystals with phosphate groups in PBS. Subsequently, the carboxylate and hydroxyl groups of TA reacted with Cu^{2+} ions in crystals to initiate the formation of TA- $Cu_3(PO_4)_2$ complexes that serve as nucleation sites for primary nanopetals. The TA-incorporated petals bound to each other, promoting their aggregation into hierarchical structures. Finally, upon saturation of anisotropic growth, a fully developed branched flower-like nanoflower structure is formed [8, 21, 22]. The formation of nucleation, coordination driven assembly and anisotropic growth occur during 3 days incubation times. During this period, the initially formed nanopetals undergo aggregation and self assembly, ultimately resulting in the flower like morphology.

Photographs of the synthesized TA NFs in dry powder and aqueous dispersed forms are presented in Figure 1A and 1B, respectively. The morphology of the synthesized TA NFs was investigated by scanning electron microscopy (SEM). As presented in Figure 1C, the TA NFs exhibited a homogeneous, spherical, and well-defined flower-like morphology. The NFs were uniformly distributed with a hierarchical architecture formed through the self-assembly of nanoscale building blocks. The average particle size was approximately 7 µm, demonstrating the successful synthesis of

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characteristic flower-like TA-NF structures. The uniform morphology and hierarchical structure are expected to provide a large specific surface area and abundant accessible active sites, which are beneficial for catalytic applications.

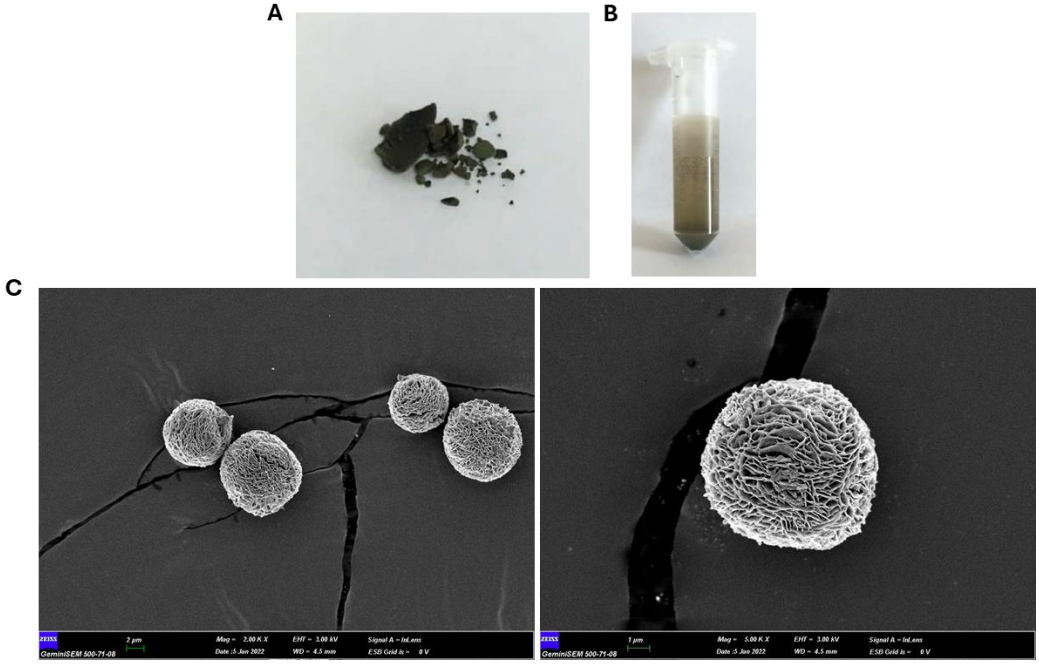

Figure 1. Photographs of the synthesized TA NFs in A) dry powder form and B) aqueous dispersed form, C) SEM images of TA NFs.

3.2. Catalytic Activity of Tannic Acid Nanoflowers

Peroxidase like activity of TA NFs was investigated by using TMB as peroxidase substrate. The peroxidase like activity is attributed to a Fenton-like reaction, as described in Equation1. According to Equation 1., Cu^{2+} ions react with H_2O_2, resulting the formation of highly reactive hydroxyl radicals ($OH^{\bullet}$) [23]. The resultant $OH^{\bullet}$ oxidize TMB substrat to its oxidized form (TMB_{ox}), which causes distinct color change from colorless to blue. The intensity of the blue coloration is proportional to the catalytic activity of the TA NFs and can be monitored spectrophotometrically.

The UV-Vis absorption spectra presented in Figure 2A demonstrated that the absorbance intensity at 652 nm, corresponding to TMB_{ox}, increased significantly as the H_2O_2 concentration increased, confirming the efficient catalytic generation of hydroxyl radicals by the TA NFs. Furthermore, the absorbance values of solutions in the presence of 500 μM H_2O_2 at 652 nm increased with the time (Figure 2B).

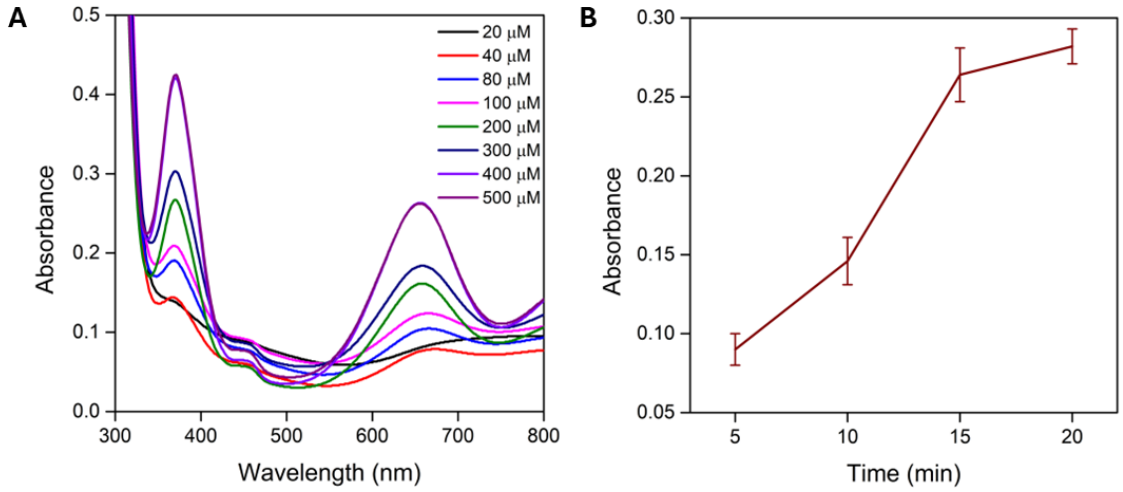

Figure 2. A) Peroxidase like activity of TA NFs, B) The absorbance values of reaction values with time in the presence of 500 μM H_2O_2.

3.3. Dye Degradation Performance of Tannic Acid Nanoflowers

TA NFs having peroxidase like activities can produce hydroxyl radicals and these radicals can degrade organic dye molecules. Herein, the degradation performance of TA NFs was investigated by using acid orange 7 (AO 7) dye. As shown in Figure 3A, the absorbance values of AO 7 + H_2O_2 at 485 nm decreased when TA NFs was added to system. This behavior can be attributed to the peroxidase-like activity of TA NFs: the catalytic centers within the NFs structure decompose H_2O_2 to generate reactive oxygen species (ROS), such as $OH^{\bullet}$, which oxidatively cleave the azo bond and the conjugated aromatic system of AO 7, leading to loss of the chromophore and thus decolorization [24]. Similarly, previous studies on nanozyme/nanomaterial systems exhibiting peroxidase-like activity have reported decolorization of azo dyes via H_2O_2 activation [25]. These results demonstrate that TA NFs can act as an effective catalyst for the oxidative removal of AO 7 by exhibiting enzyme-mimetic catalytic behavior.

The pH profile of degradation efficiency was demonstrated in Figure 3B. In the absence of TA NFs (Buffer + H_2O_2 + AO 7), the absorbance remained essentially constant across all pH, confirming that AO 7 is intrinsically stable toward H_2O_2 alone and there is no significant non-catalytic degradation occurs over the studied pH range. In contrast, in the presence of TA NFs, a clear pH-dependent decrease in absorbance was observed. The concentration of AO 7 was 30 mg L^{-1}. Specifically, the degradation efficiency was 16.5% at pH 4, increased to 24.6% at pH 7, and reached 94.0% at pH 10 in 15 min. The enhanced AO 7 degradation at high pH may be associated with pH-dependent changes in the Cu/H_2O_2 catalytic systems. Increasing pH promotes the formation of the hydroperoxide anion (HO_2^-), which can facilitate the Cu^{2+}/Cu^{+} redox cycling [32]. Furthermore, at elevated pH, deprotonation of the phenolic groups of TA may alter its electronic and redox properties, which could facilitate Cu^{2+}/Cu^{+} redox cycling and contribute to enhanced H_2O_2 activation and radicals generation [40]. Therefore, high removal efficiency of AO 7 (94%) at pH 10 may result from the enhanced Cu-mediated H_2O_2 activation.

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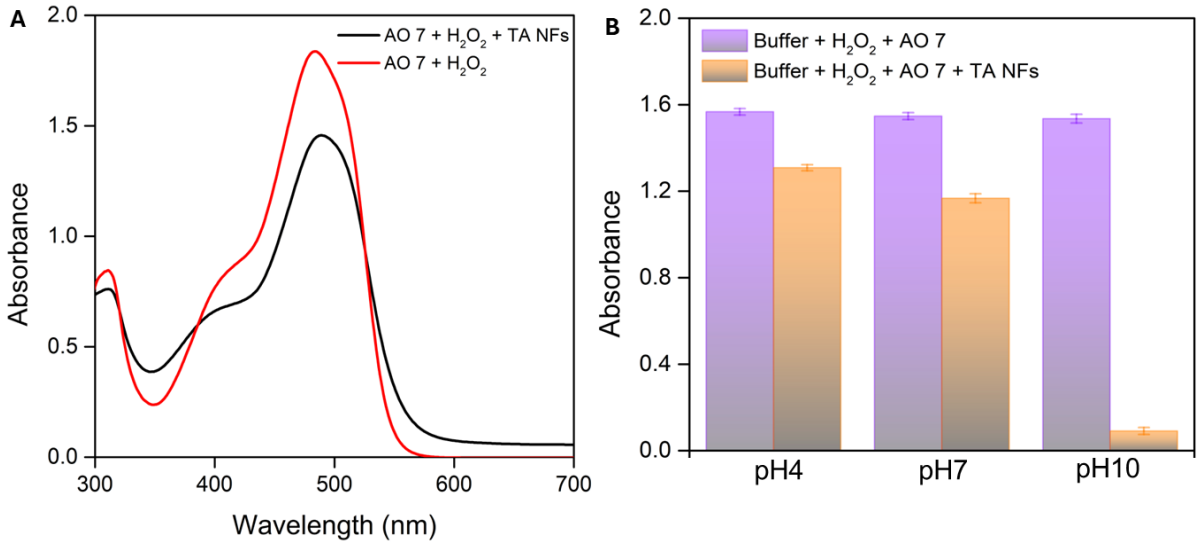

Figure 3. A) UV visible spectra of AO 7 degradation in the presence of H_2O_2 + TA NFs B) pH profile of dye degradation

The effects of different concentrations of H_2O_2 on the degradation efficiency was determined (Figure 4A). The degradation performance of TA NFs increased with increasing of H_2O_2 concentration. At pH 10, TA NFs achieved nearly 94% degradation of AO 7 (30 mg L^{-1}) in the presence of 1 mM H_2O_2. This behavior is consistent with the expected mechanism of peroxidase like catalysis: H_2O_2 acts as the co-substrate that is activated at the catalytic sites of TA NFs to generate OH•, which then attack and cleave the azo bond of AO 7. Higher H_2O_2 concentrations generate more oxidizing radicals, which speeds up AO 7 degradation, consistent with previous peroxidase and peroxidase-mimic studies [26, 27].

Finally, the effect of dye concentration on the degradation performance of TA NFs was studied by varying concentrations of 10 mg L^{-1}, 30 mg L^{-1}, 50 mg L^{-1}, and 100 mg L^{-1}. The reaction was carried out at pH 10 carbonate/bicarbonate buffer in the presence of 1 mg mL^{-1} TA NFs and 1 mM H_2O_2 for 15 min. Figure 4B shows that the degradation efficiency of TA NFs increased with increasing dye concentration. The degradation efficiency was 79% at an initial AO 7 concentration of 10 mg L^{-1}, whereas it increased to 94% and 93% at concentrations of 30 mg L^{-1} and 50 mg L^{-1}, respectively. However, when the AO 7 concentration was further increased to 100 mg L^{-1}, the degradation efficiency decreased to 76%. This decrease may be attributed to the insufficient generation of OH• relative to the higher dye concentration, resulting in reduced degradation efficiency [28].

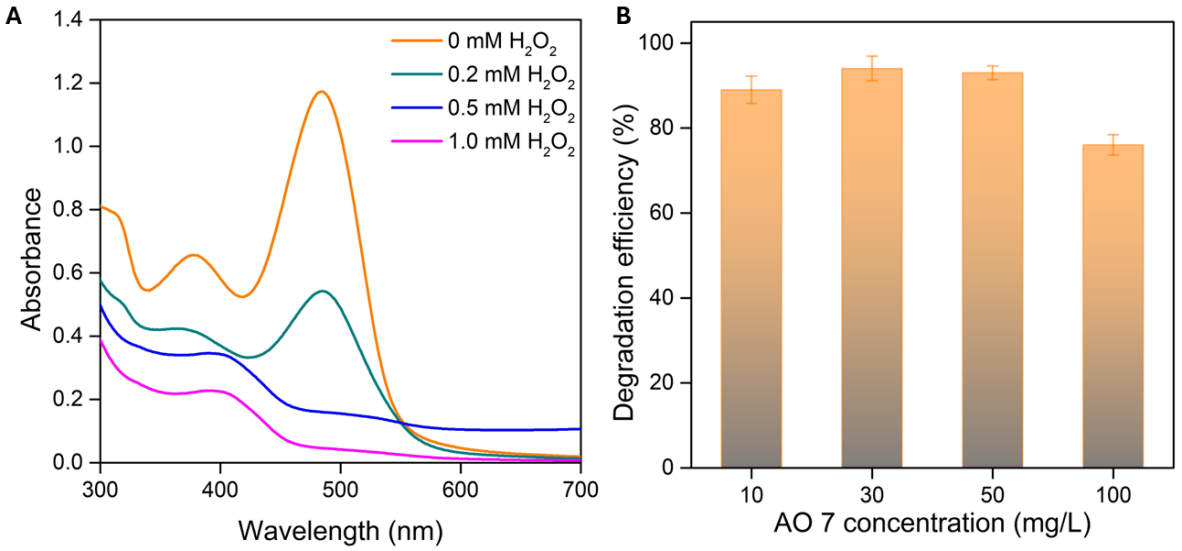

Figure 4. A) H_2O_2 concentration effect on AO 7 degradation, B) Degradation efficiency of AO. 7.

The catalytic performance of the synthesized TA NFs compares favorably with previously reported catalysts for AO 7 degradation. For example, a Co(II) TPHPP–Cs/GO nanocomposite achieved 94% degradation of AO 7 ($1.42x10^{-4}$ M) within 60 min at pH 9 [29], while a Ch-MnO_2-Ir/CeO_2 with $S_2O_8^{2-}$ system degraded 80% of 70.06 mg L^{-1} AO 7 in 100 min [30]. Similarly, converter slag iron tailings, containing 73.7% iron oxide along with trace amounts of Cu and other metal species, required 60 min to completely degrade 30 mg L^{-1} of AO 7 [31]. In contrast, the TA NFs developed in this study attained 94% decolorization within 15 min under mild reaction conditions (pH 10 and 1 mM H_2O_2), demonstrating a substantially faster degradation rate than these previously reported catalytic systems. In summary, although NFs based materials have previously been investigated for dye degradation, TA based NFs remain relatively unexplored as catalytic materials for this purpose. A particularly important feature of the developed TA NFs is their strong pH dependent catalytic behavior, with the degradation efficiency increasing from 16.5% at pH 4 and 24.6% at pH 7 to 94.0% at pH 10 within only 15 min. This pronounced catalytic activity under alkaline conditions highlights the potential of TA NFs as an efficient nanozyme like platform for azo dye degradation in textile dye-contaminated wastewater. Furthermore, the development of TA NFs is cost effective due to the use of readily available tannic acid and $CuSO_4.5H_2O$ as the organic and inorganic components, respectively, together with a simple aqueous synthesis procedure using PBS [5].

4. CONCLUSION

Organic–inorganic hybrid nanoflowers have been widely investigated as enzyme-mimicking nanomaterials owing to their high catalytic efficiency, large surface-to-volume ratio, and excellent stability. In this study, the synthesized TA NFs demonstrated intrinsic peroxidase-like activity by catalyzing the oxidation of TMB in the presence of H_2O_2. Furthermore, the TA NFs exhibited efficient dye degradation capability through a Fenton-like reaction. The degradation performance was strongly influenced by both the solution pH and the H_2O_2 concentration. Under the optimized conditions (pH 10 and 1 mM H_2O_2), the TA NFs achieved 94% decolorization of AO 7 within 15 min. These findings suggest that TA NFs represent a promising nanozyme platform for environmental remediation, particularly for the rapid degradation of azo dyes in wastewater treatment. Owing to their facile synthesis, high catalytic activity, and excellent degradation efficiency, TA NFs have considerable potential as cost-effective and sustainable catalysts for advanced oxidation processes.

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Declarations:

1. Statement of Originality:
This work is original.

2. Author Contributions:
Concept: ŞD; ***Conceptualization:*** ŞD; ***Literature Search:*** ŞD; ***Data Collection:*** ŞD; ***Data Processing:*** ŞD; ***Analysis:*** ŞD; ***Writing – original draft:*** ŞD; ***Writing – review & editing:*** ŞD.

3. Ethics approval:
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The author declares no competing interests.

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During the preparation of this work, the author used AI tools for language editing. After using this tool, the author reviewed and edited the content and takes full responsibility for the content of the publication.

7. Sustainable Development Goals:

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