

Synergistic Sonocatalytic Degradation of Sulfasalazine Using Green-Synthesized Nanoscale Zero-Valent Iron: Optimization, Kinetics, and Toxicity Assessment

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Synergistic Sonocatalytic Degradation of Sulfasalazine Using Green-Synthesized Nanoscale Zero-Valent Iron: Optimization, Kinetics, and Toxicity Assessment

Abstract

Green-synthesized nanoscale zero-valent iron (nZVI) prepared using oak leaf extract was employed as a catalyst in a sono-Fenton system (US/nZVI/H₂O₂) for the efficient degradation of sulfasalazine (SSZ). Characterization by FESEM, TEM, XRD, zeta potential, and EDS confirmed the formation of spherical core-shell nanoparticles (average size $\approx$ 32 nm) with a metallic Fe⁰ core, an iron-oxide shell, and surface-bound polyphenolic compounds. Under optimized conditions (pH 3.0, nZVI 1.5 g L⁻¹, H₂O₂ 1.5 mM, ultrasound power 150 W, 90 min), 95.8% SSZ degradation was achieved. The process followed pseudo-first-order kinetics ($k_1 = 0.0268 \text{ min}^{-1}$, $t_{1/2} = 25.9 \text{ min}$) and exhibited a clear synergistic effect among the three components. TOC removal reached 86.7% after 120 min, indicating substantial mineralization. Application to real pharmaceutical wastewater yielded 78.4% SSZ, 55.6% COD, 52.3% TOC, and 72.8% color removal. Bacterial toxicity tests showed a dramatic reduction in growth inhibition (from 89–98% to 10–21%). These results demonstrate that the green-synthesized nZVI-based sono-Fenton system is an effective and environmentally friendly technology for the treatment of SSZ-contaminated water and pharmaceutical wastewater.

Keywords: Green synthesis; Nanoscale zero-valent iron; Sono-Fenton; Sulfasalazine; Pharmaceutical wastewater; Toxicity assessment;

Introduction

The widespread occurrence of pharmaceutical contaminants in aquatic environments has become a major global environmental concern owing to their persistence, biological activity, and potential adverse effects on both ecosystems and human health [1]. Among these emerging pollutants, sulfasalazine (SSZ), a sulfonamide antibiotic extensively prescribed for the treatment of inflammatory bowel disease and rheumatoid arthritis, has attracted increasing attention because of its high consumption, incomplete metabolism in humans, and continuous release into municipal and hospital wastewater [2]. Due to its complex aromatic structure and high chemical stability, SSZ is poorly removed by conventional wastewater treatment plants, allowing it to persist in surface waters and sediments. Its long-term environmental presence contributes to the development of antibiotic-resistant microorganisms, disrupts aquatic ecosystems, and poses chronic toxicological risks to non-target organisms [3]. Consequently, the development of efficient, sustainable, and environmentally friendly technologies for SSZ removal has become an urgent research priority [4].

Conventional treatment methods, including biological degradation, adsorption, coagulation–flocculation, and membrane filtration, generally suffer from significant limitations when applied to persistent pharmaceutical compounds. Biological processes often fail because of the low biodegradability of SSZ, whereas adsorption and membrane-based technologies merely transfer contaminants from one phase to another, generating secondary waste that requires further treatment [5, 6]. These shortcomings have stimulated extensive interest in advanced oxidation processes (AOPs), which rely on the in situ generation of highly reactive oxygen species, particularly hydroxyl radicals ($\bullet\text{OH}$, $E^\circ = 2.8 \text{ V}$), capable of non-selectively oxidizing and mineralizing a wide range of refractory organic pollutants into harmless end products [7].

Among AOPs, the Fenton and Fenton-like processes are recognized as highly efficient and economically attractive oxidation technologies because of their ability to rapidly generate hydroxyl radicals through the catalytic decomposition of hydrogen peroxide [8]. Nevertheless, the conventional homogeneous Fenton process is hindered by several inherent drawbacks, including its narrow optimum pH range (approximately 2–3), excessive iron sludge generation, catalyst recovery difficulties, and considerable operational costs [9]. To overcome these limitations, nanoscale zero-valent iron (nZVI) has emerged as an effective heterogeneous Fenton catalyst. Owing to its high specific surface area, abundant reactive sites, and continuous release of Fe^{2+} ions, nZVI significantly enhances radical generation while offering magnetic recoverability and improved catalytic stability [10]. However, chemically synthesized nZVI is commonly produced using hazardous reducing agents such as sodium borohydride, increasing production costs and creating environmental concerns that compromise the sustainability of the process [11].

To address these issues, green synthesis has gained considerable attention as an environmentally benign alternative for producing iron nanoparticles. Plant extracts contain abundant phytochemicals—including polyphenols, flavonoids, tannins, and other antioxidant compounds—that simultaneously reduce iron ions to zerovalent iron and stabilize the resulting nanoparticles through surface capping, thereby minimizing aggregation and oxidation [12]. Among the available botanical resources, oak (*Quercus* spp.) leaves represent an attractive and underutilized biomass because of their high polyphenolic content, natural abundance, low cost, and renewable nature. Green-synthesized nZVI prepared from oak leaf extract therefore offers a sustainable catalyst with enhanced stability and catalytic activity while avoiding the environmental drawbacks associated with conventional synthesis routes [13].

Despite these advantages, practical application of nZVI remains constrained by nanoparticle agglomeration, gradual surface passivation, limited active-site accessibility, and reduced electron-transfer efficiency during prolonged operation [14]. Coupling nZVI with complementary physical activation techniques has emerged as an effective strategy to overcome these challenges. In particular, ultrasound (US)-assisted Fenton systems, commonly referred to as sono-Fenton processes, have demonstrated

remarkable potential for enhancing pollutant degradation. Acoustic cavitation generated by ultrasonic irradiation produces transient microbubbles that collapse violently, creating localized hotspots with extremely high temperatures and pressures [15]. These extreme conditions promote the homolytic cleavage of water molecules and hydrogen peroxide, generating additional hydroxyl radicals while simultaneously improving catalyst dispersion, removing passivating surface layers, preventing nanoparticle aggregation, and enhancing mass transfer between pollutants and catalytic active sites [16]. Consequently, the combination of ultrasound, hydrogen peroxide, and green-synthesized nZVI creates multiple radical-generation pathways that substantially improve oxidation efficiency compared with the individual processes.

Although several studies have investigated plant-mediated synthesis of nZVI or the application of sono-Fenton oxidation independently, studies integrating oak leaf-derived green nZVI with ultrasound-assisted Fenton oxidation for sulfasalazine degradation remain scarce [17, 18]. Furthermore, comprehensive investigations addressing the mechanistic interactions responsible for the observed synergistic effects, degradation pathways, catalyst stability, mineralization efficiency, applicability to real wastewater matrices, and reduction of residual toxicity are still limited. Addressing these knowledge gaps is essential for evaluating the practical feasibility of this sustainable treatment technology.

Therefore, the present study aims to develop and evaluate an environmentally friendly oak leaf-mediated green-synthesized nZVI/US/H₂O₂ sono-Fenton system for the degradation of sulfasalazine in aqueous media. The specific objectives are to: (i) synthesize and characterize green nZVI using oak leaf extract; (ii) optimize the operational parameters governing SSZ degradation; (iii) investigate degradation kinetics and quantify the synergistic interactions among ultrasound, nZVI, and hydrogen peroxide; (iv) evaluate mineralization efficiency and performance in real wastewater; and (v) assess the reduction of residual toxicity using a bacterial growth inhibition assay.

The findings provide mechanistic insights into sustainable sono-Fenton catalysis and demonstrate the potential of biomass-derived green nZVI as an efficient and environmentally benign catalyst for the treatment of pharmaceutical-contaminated wastewater.

Materials and Methods

Chemicals and Reagents

Sulfasalazine (SSZ, C₁₈H₁₄N₄O₅S, molecular weight = 398.39 g/mol, purity ≥ 98%, λ_{max} = 359 nm) was obtained from Sigma-Aldrich (USA). Ferrous sulfate heptahydrate (FeSO₄·7H₂O, ≥ 99%), hydrogen peroxide (H₂O₂, 30% w/w), sodium hydroxide (NaOH, ≥ 98%), hydrochloric acid (HCl, 37%), and sulfuric acid (H₂SO₄, 98%) were purchased from Merck (Germany) and used without further purification. HPLC-grade acetonitrile and acetic acid were also obtained from Merck (Germany). All solutions were

prepared using deionized water (resistivity = 18.2 MΩ·cm) from a Milli-Q water purification system (Millipore, France). A stock solution of SSZ (1000 mg/L) was prepared in deionized water and stored in amber glass bottles at 4 °C in the dark. Working solutions (1–100 mg/L) were freshly prepared by dilution of the stock solution immediately before each experiment.

Preparation of Oak Leaf Extract

Fresh oak leaves (*Quercus brantii*) were collected during the spring season from the Zagros forests in Nurabad Mamasani, Fars Province, Iran. Only healthy, mature leaves with uniform green coloration were selected. The leaves were thoroughly washed with tap water, rinsed three times with distilled water, and shade-dried at room temperature (25 ± 2 °C) for 72 h. The dried leaves were ground into a fine powder using an electric grinder and passed through a 0.5 mm sieve. The powdered material was stored in airtight containers at 4 °C in the dark until use.

For extract preparation, 100 g of dried oak leaf powder was added to 1000 mL of deionized water and heated at 60 °C in a water bath with continuous magnetic stirring (500 rpm) for 30 min. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper under vacuum. The filtrate was collected in amber glass bottles, stored at 4 °C in the dark, and used within 7 days of preparation.

Green Synthesis of Nanoscale Zero-Valent Iron (nZVI)

Green synthesis of nZVI was performed according to an optimized protocol. Briefly, 27.8 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was dissolved in 500 mL of deionized water to obtain a 0.2 M iron solution. The iron solution was mixed with oak leaf extract at a volume ratio of 2:3 (200 mL iron solution : 300 mL extract) under continuous magnetic stirring (600 rpm) at room temperature. The pH of the mixture was adjusted to 6.0 using 0.1 M H_2SO_4 or NaOH. The reaction was allowed to proceed for 1 h, during which the solution color changed from yellow-brown to black, indicating the formation of nZVI.

The black precipitate was collected by centrifugation (10000 rpm, 10 min), washed three times with deionized water and twice with ethanol, and dried in a vacuum oven at 60 °C for 24 h. The dried product was ground in an agate mortar, sieved through a 200-mesh screen, and stored under argon atmosphere in sealed vials. The synthesized nZVI was characterized within 48 h of preparation.

Characterization of nZVI

Surface morphology was examined by field-emission scanning electron microscopy (FESEM, MIRA3 TESCAN-XMU) operated at 15 kV. Particle size and morphology were determined by transmission electron microscopy (TEM, Philips CM120) at 100 kV; particle size distribution was calculated by

measuring at least 200 particles using ImageJ software. Crystalline structure was analyzed by X-ray diffraction (XRD, Philips X'Pert MPD) with Co K α radiation ($\lambda = 1.78897 \text{ \AA}$) in the 2θ range of $5\text{--}80^\circ$. Average crystallite size was calculated using the Debye–Scherrer equation. Surface functional groups were identified by Fourier-transform infrared spectroscopy (FTIR, Bruker Tensor 27) using the KBr pellet method in the range of $4000\text{--}400 \text{ cm}^{-1}$. Zeta potential was measured with a Zetasizer Nano ZS (Malvern Instruments) in the pH range of $3\text{--}11$, and the point of zero charge (pH_{pzc}) was determined. Elemental composition was analyzed by energy-dispersive X-ray spectroscopy (EDS) coupled to the FESEM system.

Sonocatalytic Degradation Experiments

Degradation experiments were conducted in a jacketed glass batch reactor (500 mL capacity) equipped with an ultrasonic probe (Qsonica Q700, USA) operating at 20 kHz with adjustable power (0–500 W). The reactor temperature was maintained at $25 \pm 1^\circ\text{C}$ using a circulating water bath. The reaction volume was 250 mL in all experiments.

In each run, 250 mL of SSZ solution (desired concentration) was placed in the reactor and the pH was adjusted ($3\text{--}11$) using 0.1 M H_2SO_4 or NaOH. A specified amount of nZVI (0.05–3.0 g/L) was added, and the suspension was stirred at 500 rpm for 30 min in the dark to reach adsorption–desorption equilibrium. After sampling ($t = 0$), the required amount of H_2O_2 (0.05–5 mM) was added and ultrasound irradiation (40–200 W) was started with the probe immersed 2 cm below the solution surface. Aliquots (5 mL) were withdrawn at predetermined intervals, immediately filtered through $0.22 \mu\text{m}$ syringe filters, and stored at 4°C until analysis. Control experiments were performed without ultrasound (nZVI/ H_2O_2) and without nZVI (US/ H_2O_2). All experiments were conducted in triplicate.

Analytical Methods

SSZ concentration and degradation intermediates were quantified by high-performance liquid chromatography (HPLC) using a Shimadzu LC-20AD system equipped with a UV-Vis detector (SPD-20A). Separation was achieved on a C18 reversed-phase column ($250 \text{ mm} \times 4.6 \text{ mm}$, $5 \mu\text{m}$, Phenomenex) with a mobile phase of acetonitrile:water:acetic acid (30:70:1, v/v/v) at a flow rate of 1.0 mL/min. Detection was performed at 254 nm, the injection volume was $20 \mu\text{L}$, and the column temperature was maintained at 30°C . The retention time of SSZ was approximately 4.2 min. Calibration curves were constructed in the range of 0.5–50 mg/L ($R^2 = 0.999$). The limit of detection (LOD) and limit of quantification (LOQ) were 0.08 mg/L and 0.25 mg/L, respectively. The method was validated for linearity, precision, accuracy, and selectivity according to ICH Q2(R1) guidelines. All samples were

filtered through 0.22 µm syringe filters prior to injection. Degradation efficiency was calculated from the reduction in SSZ peak area.

Mineralization efficiency was evaluated by total organic carbon (TOC) analysis using an Analytik Jena multi N/C 2100 analyzer with the Non-Purgeable Organic Carbon (NPOC) method. Samples were acidified to pH < 2 and purged to remove inorganic carbon. TOC removal was calculated as:

$$\text{TOC removal (\%)} = \frac{\text{TOC}_0 - \text{TOC}_t}{\text{TOC}_0} \times 100$$

Chemical oxygen demand (COD) was determined by the closed reflux colorimetric method according to Standard Methods. Analysis was performed using a Hach DRB 200 COD reactor and Hach DR 6000 spectrophotometer. Samples were filtered through 0.45 µm filters prior to analysis.

Kinetics and Synergy Study

The degradation kinetics was analyzed using pseudo-first-order and pseudo-second-order models. The pseudo-first-order model is expressed as:

$$\ln\left(\frac{C_0}{C_t}\right) = k_1 t$$

where k_1 is the pseudo-first-order rate constant (min^{-1}). The pseudo-second-order model is expressed as:

$$\frac{1}{C_t} = \frac{1}{C_0} + k_2 t$$

where k_2 is the pseudo-second-order rate constant ($\text{L} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$). The correlation coefficient (R^2) was used to evaluate the goodness of fit. The goodness of fit was evaluated by the linear correlation coefficient (R^2). The half-life for the pseudo-first-order reaction was calculated as $t_{1/2} = \ln 2 / k_1$.

$$t_{1/2} = \frac{\ln 2}{k_1}$$

Synergistic effects were evaluated under seven systems: (1) US alone, (2) nZVI alone, (3) H_2O_2 alone, (4) US + nZVI, (5) US + H_2O_2 , (6) nZVI + H_2O_2 , and (7) US + nZVI + H_2O_2 . All experiments were performed under optimal conditions (pH = 3.0, nZVI = 1.5 g/L, H_2O_2 = 1.5 mM, US power = 150 W, SSZ = 50 mg/L). The synergy ratio (SR) was calculated as:

$$SR = \frac{k_{\text{app(combined)}}}{k_{\text{app(US)}} + k_{\text{app(nZVI)}} + k_{\text{app(H}_2\text{O}_2\text{)}} + k_{\text{app(US + nZVI)}} + k_{\text{app(US + H}_2\text{O}_2\text{)}} + k_{\text{app(nZVI + H}_2\text{O}_2\text{)}} + k_{\text{app(nZVI)US + nZVI + H}_2\text{O}_2}}$$

A value of $SR > 1$ indicates a synergistic effect.

Real Wastewater Treatment

Real wastewater samples were collected from a pharmaceutical manufacturing facility. The samples were characterized for pH, COD, TOC, color, TSS, TDS, and electrical conductivity according to Standard Methods. Treatment was performed under optimal conditions (pH = 3.0, nZVI = 1.5 g/L, H₂O₂ = 1.5 mM, US power = 150 W) for 90 min, and removal efficiencies for SSZ, COD, TOC, and color were determined.

Matrix effects were investigated by adding chloride (500 mg/L), sulfate (300 mg/L), carbonate (200 mg/L), nitrate (100 mg/L), or humic acid (20 mg/L) to synthetic SSZ solutions at concentrations typical of real wastewater. Degradation efficiency was measured after 60 min.

Toxicity Assessment

The residual toxicity of untreated and treated sulfasalazine solutions was evaluated using a bacterial growth inhibition (GI%) assay against six standard strains: four Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Haemophilus influenzae* ATCC 49247, and *Salmonella enterica* ATCC 19430) and two Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Streptococcus pneumoniae* ATCC 49619).

Each strain was cultured in Mueller–Hinton broth at 37 °C with shaking at 150 rpm. The optical density was adjusted to OD₆₀₀ = 0.1 (approximately 10⁸ CFU mL⁻¹). Untreated and treated SSZ solutions (final concentration 50 mg L⁻¹) were added to the bacterial cultures in sterile 96-well microplates and incubated at 37 °C for 12 h. OD₆₀₀ was recorded at 2-hour intervals. A negative control (bacterial culture without SSZ) and a positive control (culture containing 10 µg mL⁻¹ ampicillin) were included in each experiment.

Growth inhibition was calculated according to the following equation [19]:

$$GI\% = \left(\frac{OD_{600S}}{OD_{600C}} - 1 \right)$$

where OD_{600S} and OD_{600C} are the optical densities of the sample and the negative control, respectively. All experiments were performed in triplicate.

Results and Discussion

Characterization of Green-Synthesized nZVI

Structural and Morphological Characterization

The surface morphology and microstructure of the fresh and spent green-synthesized nZVI were examined using field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). Fig. 1 presents the SEM and TEM micrographs of both fresh and spent nZVI,

providing insight into the morphological and structural changes that occur during the sonocatalytic degradation process.

Fig. 1A shows the SEM image of fresh nZVI. The particles exhibit a predominantly spherical to sub-spherical morphology with a relatively uniform size distribution and form chain-like aggregates. This aggregation is characteristic of magnetic nanoparticles and arises from magnetic dipole–dipole interactions together with the presence of a surface iron-oxide shell [20]. Such aggregation is commonly observed for green-synthesized nZVI and does not preclude catalytic activity, because reactivity is governed by the accessibility of surface active sites rather than the degree of dispersion [21]. The particle surface appears relatively smooth with only minor roughness, consistent with the presence of residual polyphenolic compounds from the oak-leaf extract that act as capping agents [13]. Particle sizes estimated from FESEM images range from approximately 30–80 nm, with the majority falling between 40 and 60 nm.

After sonocatalytic degradation of sulfasalazine (Fig. 1B), the spent nZVI displays clear morphological changes. Although a roughly spherical shape is partially retained, the particles become more aggregated and the surface is markedly rougher, with visible deposits of organic residues and iron-oxide layers. The increased aggregation is attributed to surface oxidation and the adsorption of degradation intermediates and by-products during the sono-Fenton process [22]. The observed surface roughness is indicative of progressive corrosion and the formation of iron oxide/hydroxide species, which are typical of Fenton-like reactions involving nZVI [23].

Fig. 1C shows the TEM image of fresh nZVI. The nanoparticles are spherical and possess a distinct core–shell structure: the dark core corresponds to metallic Fe^0 and the lighter outer layer to iron oxides ($\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$) formed by surface oxidation during synthesis and storage. This core–shell architecture is advantageous for Fenton-like catalysis because the oxide shell supplies active sites for radical generation while the metallic core acts as an electron reservoir, thereby sustaining catalytic activity [24]. The average particle diameter determined from TEM images of fresh nZVI is 32 ± 8 nm ($n = 200$), in agreement with the FESEM observations. High-resolution TEM reveals lattice fringes with an interplanar spacing of 0.203 nm, corresponding to the (110) plane of body-centered-cubic (bcc) α -Fe (JCPDS 06-0696). An amorphous shell approximately 2–3 nm thick surrounds the crystalline core, consistent with a thin iron-oxide layer [25].

After 40 min of sonocatalytic degradation (Fig. 1D), the spent nZVI exhibits significant structural alteration. The core–shell morphology is still discernible, but the oxide shell is thicker and more irregular. The average particle size increases to approximately 45 ± 10 nm, reflecting surface oxidation and deposition of reaction products. Lattice fringes of the residual Fe^0 core become less distinct in places, indicating partial oxidation of the metallic core. Additional amorphous surface layers are ascribed to

adsorbed organic intermediates and precipitated iron hydroxide species formed during the sono-Fenton reaction. These changes are consistent with progressive consumption of the Fe⁰ core and conversion of the nanoparticle surface to iron oxide/hydroxide phases [26].

Crystalline Structure Analysis

The crystalline structure and phase composition of fresh and spent nZVI were examined by X-ray diffraction (XRD). Fig. 2 shows the XRD patterns of both samples and illustrates the structural transformations that accompany the sono-Fenton reaction.

The XRD pattern of fresh nZVI (Fig. 2A) displays the characteristic reflections of bcc α -Fe at approximately 45.0° and 65.5° 2 θ , corresponding to the (110) and (200) planes (JCPDS 06-0696). Broader features in the regions ~20–25° and ~30–40° are consistent with the presence of iron-oxide phases (Fe₂O₃/Fe₃O₄) on the particle surface and confirm the core–shell structure observed by TEM. The diffraction peaks are relatively broad, as expected for nanocrystalline material. Application of the Debye–Scherrer equation to the (110) reflection (FWHM $\approx$ 2.05°) yields an average crystallite size of 4.2 ± 0.3 nm. This value is substantially smaller than the overall particle diameter measured by TEM (~32 nm), indicating that each nanoparticle is polycrystalline and composed of multiple crystallites. The high density of grain boundaries and defects associated with such nanocrystalline material provides additional active sites for electron transfer and surface-mediated reactions, enhancing catalytic performance [27].

The XRD pattern of the spent catalyst (Fig. 2B) shows a clear decrease in the intensity of the metallic Fe⁰ reflections at ~45° and ~65°, indicating partial consumption of the metallic core. Concurrently, the relative intensity of the iron-oxide contributions increases, consistent with progressive oxidation of Fe⁰. Peak broadening of the oxide features suggests the formation of smaller crystallites or the presence of amorphous iron oxide/hydroxide phases. The crystallite size of the residual Fe⁰, calculated from the (110) peak, decreases slightly to 3.8 ± 0.3 nm. These XRD results corroborate the TEM observations and demonstrate the transformation of nZVI from a metallic-core/oxide-shell architecture toward a more extensively oxidized state during the sono-Fenton process [28].

Zeta Potential and EDS Analysis

Zeta-potential measurements as a function of pH (Fig. 3A) yield a point of zero charge (pH_{pzc}) of 6.8 ± 0.3 for the green-synthesized nZVI. Below pH 6.8 the surface is positively charged (protonation of surface hydroxyl groups, Fe–OH₂⁺); above pH 6.8 it is negatively charged (deprotonation, Fe–O[–]). At pH 3 the zeta potential reaches approximately +38 mV, reflecting a strongly positive surface that provides electrostatic stabilization against aggregation. The high positive potential under acidic conditions arises from protonation of both iron-oxide surface sites and residual polyphenolic compounds. Absolute zeta-

potential values $|\zeta| > 30$ mV over a broad pH range (approximately 3–9) indicate good colloidal stability, which is advantageous for catalytic performance [29].

Energy-dispersive X-ray spectroscopy (EDS) analysis (Fig. 3B) confirms the presence of C, O, S, Cl and Fe as the principal elemental constituents. Quantitative results show an iron content of 11.77 wt% (3.53 at%), verifying successful formation of nZVI. The high oxygen content (50.03 wt%, 52.43 at%) is consistent with an iron-oxide shell ($\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$) surrounding a metallic core, in agreement with the TEM observations. The Fe/O weight ratio of ~ 0.235 further supports this core-shell structure. Carbon (27.57 wt%, 38.49 at%) originates from residual polyphenolic compounds of the oak-leaf extract that act as capping and stabilizing agents [39]. Sulfur (10.30 wt%, 5.38 at%) is attributable to residual sulfate from the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ precursor, while the very low chlorine content (0.33 wt%, 0.16 at%) indicates effective washing of the product [30].

Optimization of Degradation Parameters

Effect of pH

Solution pH exerted the strongest influence on SSZ degradation in the US/nZVI/ H_2O_2 system (Fig. 4A). Efficiency decreased sharply from 95.8% at pH 3 to 28.7% at pH 11, reflecting the well-known pH sensitivity of Fenton chemistry.

At strongly acidic conditions (pH 2–4), Fe^{2+} ions remain soluble and highly reactive, allowing rapid generation of hydroxyl radicals through the classical Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \bullet\text{OH}$). Concurrently, the nZVI surface is protonated ($\text{Fe}-\text{OH}_2^+$), producing a positive charge that electrostatically attracts the negatively charged SSZ molecules (carboxylic acid group, $\text{pK}_a \approx 2.4$). This dual effect—high radical production and enhanced pollutant adsorption—maximizes degradation efficiency. Acoustic cavitation under acidic conditions further contributes to radical generation via thermal dissociation of water molecules, while the low pH suppresses radical recombination [31].

As pH increases toward neutrality, progressive hydrolysis and precipitation of $\text{Fe}(\text{OH})_2$ and $\text{Fe}(\text{OH})_3$ remove active iron species from the solution. Near the point of zero charge ($\text{pH}_{\text{pzc}} \approx 6.8$), the surface charge approaches zero, leading to increased nanoparticle aggregation and a reduction in available active surface area [32]. Under alkaline conditions (pH 9–11), hydroxyl radicals are scavenged by OH^- ions to form the less reactive oxide radical anion ($\bullet\text{OH} + \text{OH}^- \rightarrow \text{O}^{\bullet-} + \text{H}_2\text{O}$). The oxidation potential of $\text{O}^{\bullet-}$ ($E^\circ = 1.34$ V) is substantially lower than that of $\bullet\text{OH}$ ($E^\circ = 2.8$ V), resulting in markedly diminished oxidative capacity [46]. The optimum at pH 3 is consistent with the majority of reported nZVI-based and green-synthesized nZVI Fenton systems, which typically operate most effectively in the range pH 2.5–4 [33].

Effect of nZVI Dosage

The influence of nZVI dosage displayed a clear optimum: degradation efficiency increased with catalyst loading up to 1.5 g/L and declined at higher concentrations (Fig. 4B).

At dosages below 1.5 g/L, the rise in efficiency is attributable to the greater number of active sites available for Fe^{2+} release through surface corrosion of Fe^0 ($\text{Fe}^0 + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^-$ and related reactions). The liberated Fe^{2+} subsequently drives the Fenton reaction, increasing the steady-state concentration of $\bullet\text{OH}$ [34]. The relatively high optimal dosage compared with many chemically synthesized nZVI systems (often 0.1–0.8 g/L) is explained by the modest iron content of the green-synthesized material (≈ 11.8 wt% Fe) together with the presence of residual organic matter and an iron-oxide shell. Continuous surface cleaning and reactivation by ultrasound help maintain catalyst activity within this dosage range [35].

Above 1.5 g/L, several detrimental processes become dominant: excess Fe^{2+} scavenges hydroxyl radicals ($\text{Fe}^{2+} + \bullet\text{OH} \rightarrow \text{Fe}^{3+} + \text{OH}^-$), increased solution turbidity attenuates ultrasound propagation, and enhanced particle aggregation reduces the effective surface area [36]. Collectively, these effects lower the net availability of $\bullet\text{OH}$ and decrease overall degradation efficiency.

Effect of H_2O_2 Concentration

Hydrogen peroxide concentration strongly affected SSZ removal (Fig. 4C). Efficiency rose from 38.5% at 0.05 mM to a maximum of 95.8% at 1.5 mM, then declined to 64.2% at 5.0 mM.

In the ascending portion of the curve, additional H_2O_2 increases $\bullet\text{OH}$ production through both the classical Fenton reaction and the sonochemical dissociation of H_2O_2 inside cavitation bubbles ($\text{H}_2\text{O}_2 + \text{energy} \rightarrow 2\bullet\text{OH}$). The combination of these two pathways elevates the steady-state radical concentration and enhances pollutant oxidation [37].

Beyond the optimum, excess H_2O_2 acts primarily as a radical scavenger ($\text{H}_2\text{O}_2 + \bullet\text{OH} \rightarrow \text{HO}_2\bullet + \text{H}_2\text{O}$; $\text{HO}_2\bullet + \bullet\text{OH} \rightarrow \text{H}_2\text{O} + \text{O}_2$). Additional losses occur through radical recombination and the formation of less-reactive ferric hydroperoxy complexes that hinder the regeneration of Fe^{2+} . The identified optimum of 1.5 mM falls within the range commonly reported for nZVI-assisted sono-Fenton systems (1.0–2.0 mM) [38].

Effect of Ultrasound Power

Ultrasound power was varied between 40 and 200 W (Fig. 4D). Degradation efficiency increased from 62.3% at 40 W to a maximum of 95.8% at 150 W, and then decreased to 85.6% at 200 W.

Moderate increases in power intensify acoustic cavitation, producing a larger number of cavitation bubbles and more energetic collapses. The resulting high local temperatures and pressures enhance radical generation from both water and H_2O_2 . In parallel, physical effects of ultrasound—microstreaming, acoustic streaming, nanoparticle deagglomeration, and continuous surface cleaning—improve mass transfer and help maintain catalyst accessibility [39].

At excessively high power (200 W), larger cavitation bubbles form and collapse less efficiently, the solution becomes progressively degassed (reducing the number of cavitation nuclei), and radical recombination is accelerated. These factors collectively reduce the net yield of $\bullet\text{OH}$ [40]. An optimum near 150 W is consistent with values reported for other sono-Fenton processes.

Effect of Initial SSZ Concentration

Degradation efficiency declined progressively from 98.5% at 1 mg/L to 71.2% at 100 mg/L (Fig. 4E). With fixed amounts of catalyst (1.5 g/L) and oxidant (1.5 mM), the total quantity of hydroxyl radicals that can be generated is limited. Consequently, as the number of SSZ molecules increases, a smaller fraction of the pollutant is oxidized.

Additional contributing factors include competitive consumption of $\bullet\text{OH}$ by a growing pool of degradation intermediates, partial absorption of ultrasonic energy by the SSZ molecules themselves (internal filtering effect), and progressive saturation of the limited nZVI surface area [41]. A gradual decrease in solution pH caused by the acidic nature of SSZ may also shift the system slightly away from the optimal pH of 3 [42]. These effects are reflected in the decline of the apparent pseudo-first-order rate constant from 0.0485 min^{-1} at 1 mg/L to 0.0123 min^{-1} at 100 mg/L [43].

Effect of Reaction Time

The temporal profile of SSZ degradation revealed three distinct stages (Fig. 4F). In the initial rapid phase (0–30 min), approximately 72% removal was achieved while the catalyst surface remained fresh, H_2O_2 concentration was highest, and radical generation was most efficient.

During the subsequent gradual phase (30–90 min), residual parent molecules together with more refractory intermediates formed after chromophore cleavage were oxidized, raising overall efficiency to 95.8% [44]. Beyond 90 min a plateau was observed, with only marginal further improvement ($\approx 96.5\%$ at 120 min), indicating that degradation of the parent compound was essentially complete. The 90-minute contact time identified as optimal is in good agreement with previously reported nZVI-based sono-Fenton systems [43].

Synergistic Effects

The contribution of each component and the synergistic interactions within the US/nZVI/ H_2O_2 system were assessed by comparing the degradation efficiencies of individual, binary, and ternary processes after 90 min (Fig. 5).

These results confirm that none of the components alone is capable of achieving effective SSZ degradation. Ultrasound alone generates a limited quantity of hydroxyl radicals through

sonolysis of water. nZVI alone removes SSZ primarily by adsorption rather than oxidative degradation. Direct oxidation by H_2O_2 without catalytic activation is also inefficient [45].

In the US + nZVI system, ultrasound continuously cleans the catalyst surface, improves mass transfer, and disperses nanoparticle aggregates, thereby increasing the number of accessible active sites. In the US + H_2O_2 system, cavitation enhances the thermal dissociation of hydrogen peroxide ($\text{H}_2\text{O}_2 \rightarrow 2\bullet\text{OH}$), elevating the yield of hydroxyl radicals. The nZVI + H_2O_2 combination operates as a classical heterogeneous Fenton-like system in which nZVI continuously releases Fe^{2+} that reacts with H_2O_2 to generate $\bullet\text{OH}$ [10].

The ternary system (US + nZVI + H_2O_2) achieved the highest degradation efficiency of 95.8%, markedly surpassing the performance of any binary combination and the arithmetic sum of the individual processes. The calculated synergy ratio (SR) was greater than 1, confirming a clear synergistic interaction among the three components. This synergy arises from the complementary roles of ultrasound (continuous surface activation and additional radical generation), nZVI (sustained supply of Fe^{2+}), and H_2O_2 (primary source of $\bullet\text{OH}$), which together maintain a high steady-state concentration of reactive species throughout the reaction [46].

Kinetic Analysis

The degradation kinetics of SSZ in the US/nZVI/ H_2O_2 system under optimal conditions were evaluated using both pseudo-first-order and pseudo-second-order models (Fig. 6).

Fig. 7A shows the plot of $\ln(C_0/C_t)$ versus time. A strong linear correlation was obtained ($R^2 = 0.9905$), yielding a pseudo-first-order rate constant of $k_1 = 0.0268 \text{ min}^{-1}$. The corresponding half-life was 25.9 min.

The positive intercept (0.140) indicates a short initial rapid-degradation phase, which is commonly attributed to the high reactivity of freshly available surface sites on nZVI and the immediate generation of reactive species at the beginning of the reaction [47].

Fig. 7B presents the corresponding pseudo-second-order plot ($1/C_t$ versus time). The linear fit gave $R^2 = 0.9741$ and $k_2 = 0.0020 \text{ L mg}^{-1} \text{ min}^{-1}$.

Although the second-order model provided an acceptable fit, its correlation coefficient was lower than that of the first-order model. Therefore, the degradation of SSZ is better described by pseudo-first-order kinetics.

The superiority of the pseudo-first-order model indicates that the concentration of hydroxyl radicals remains approximately constant (steady-state) during the reaction. This behavior is characteristic of continuous radical-generation systems such as Fenton and sono-Fenton processes, in which reactive oxygen species are continuously supplied by the catalyst and ultrasound [7]. The high R^2 value also suggests that external mass-transfer limitations and significant catalyst deactivation are negligible under the optimized conditions.

The obtained rate constant ($k_1=0.0268 \text{ min}^{-1}$) is comparable to or higher than values reported for many similar sono-Fenton systems treating pharmaceutical pollutants. The relatively high rate can be ascribed to the small crystallite size of the green-synthesized nZVI (4.2 nm), its high specific surface area, and the presence of residual polyphenolic compounds that facilitate electron transfer [44, 48]. The short half-life of 25.9 min further highlights the efficiency of the combined ultrasound/nZVI/ H_2O_2 process compared with conventional treatment methods that often require several hours to achieve similar removal efficiencies

TOC Removal and Mineralization Efficiency

The mineralization efficiency of the US/nZVI/ H_2O_2 system was evaluated by monitoring total organic carbon (TOC) removal alongside SSZ degradation (Fig. 7A). While SSZ degradation reached 95.8% within 90 min, TOC removal progressed more slowly and attained 86.7% only after 120 min. This temporal gap between parent-compound degradation and mineralization is characteristic of advanced oxidation processes and indicates that the initial cleavage of the SSZ molecule produces organic intermediates that require additional oxidation to reach complete conversion to CO_2 . At 90 min, TOC removal was only 58.5%, confirming that a substantial fraction of the original organic carbon remained in the form of partially oxidized intermediates. Extending the reaction to 120 min increased TOC removal to 86.7%, demonstrating progressive mineralization. The TOC-removal profile exhibited two stages: a relatively rapid phase (0–60 min) in which TOC removal rose to approximately 45%, corresponding to the breakdown of SSZ and the formation of more readily oxidizable smaller molecules, followed by a slower phase (60–120 min) during which recalcitrant intermediates were gradually mineralized.

The residual TOC of 13.3% after 120 min is most likely attributable to the presence of short-chain carboxylic acids (e.g., formic, acetic and oxalic acids) that are known to be resistant to further oxidation under mild Fenton conditions, or to a minor fraction of organic matter adsorbed

on the catalyst surface. Overall, the high degree of mineralization (86.7%) confirms the effectiveness of the sono-Fenton system for the near-complete destruction of both the parent pollutant and its organic by-products [43].

Real Pharmaceutical Wastewater Treatment and Matrix Effects

Wastewater Characterization

The characteristics of the real pharmaceutical wastewater collected from the manufacturing facility are summarized in Table 1. The wastewater exhibited a high organic load, appreciable residual SSZ concentration, intense color, and elevated levels of dissolved salts, which could potentially interfere with the advanced oxidation process.

Treatment Performance

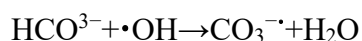
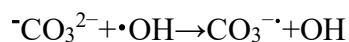
When the real wastewater was treated under the previously optimized conditions (pH 3.0, nZVI 1.5 g L⁻¹, H₂O₂ 1.5 mM, ultrasound power 150 W, 90 min), the following removal efficiencies were obtained (Figure 7B): SSZ 78.4%, COD 55.6%, TOC 52.3% and color 72.8%. The lower SSZ removal compared with the synthetic solution (95.8%) is attributable to the complex matrix of the real effluent, which contains inorganic anions, natural organic matter and other co-existing organic compounds that compete for hydroxyl radicals or interact with the catalyst surface.

Influence of Interfering Species

A systematic matrix-effect study was performed by adding individual interfering components to a synthetic SSZ solution. The observed degradation efficiencies and the corresponding reductions relative to the control (95.8%) were:

- Chloride (Cl⁻): 78.4% (-17.4%)
- Sulfate (SO₄²⁻): 81.8% (-14.0%)
- Carbonate (CO₃²⁻): 62.3% (-33.5%)
- Nitrate (NO₃⁻): 87.7% (-8.1%)
- Humic acid: 68.5% (-27.3%)

Carbonate ions produced the strongest inhibition. Both carbonate and bicarbonate act as efficient scavengers of hydroxyl radicals according to the following reactions:



The resulting carbonate radical ($\text{CO}_3^{\cdot-}$) possesses a considerably lower oxidation potential ($E^\circ \approx 1.78 \text{ V}$) than $\cdot\text{OH}$ ($E^\circ = 2.8 \text{ V}$) and is therefore far less effective at oxidizing organic pollutants. Humic acid exerted the second-strongest negative effect. It competes with SSZ for $\cdot\text{OH}$ radicals, absorbs a fraction of the ultrasonic energy (thereby reducing cavitation efficiency), and can form surface complexes with nZVI that block active sites [49].

Chloride ions scavenge $\cdot\text{OH}$ to generate chlorine radicals ($\text{Cl}^\cdot$ and $\text{Cl}_2^{\cdot-}$) of lower oxidizing power. Sulfate ions reduce the availability of free $\text{Fe}^{2+}/\text{Fe}^{3+}$ by forming complexes, while nitrate shows only a mild inhibitory effect [50].

Despite these matrix interferences, the US/nZVI/ H_2O_2 system still achieved substantial removal of SSZ, COD, TOC and color from the real pharmaceutical wastewater, demonstrating acceptable robustness for practical application.

Toxicity Assessment

The ecotoxicological safety of the sonocatalytic process was assessed by comparing the antibacterial activity of untreated and treated SSZ solutions against six standard bacterial strains (Fig.8 and Table 2).

The untreated SSZ solution (50 mg L^{-1}) exhibited strong antimicrobial activity, with growth inhibition (GI%) values ranging from 89% to 98%. The highest inhibition was observed for *Staphylococcus aureus* (98%), followed by *Haemophilus influenzae* (95%) and *Escherichia coli* (93%), while *Salmonella enterica* showed the lowest inhibition (89%). These results are consistent with the known antibacterial mechanism of sulfasalazine as a sulfonamide antibiotic that interferes with bacterial folate synthesis. The moderate variation among strains can be attributed to differences in cell-wall structure, membrane permeability, and intrinsic resistance mechanisms. Gram-negative bacteria generally displayed slightly lower GI% values than Gram-positive strains, most likely because of the protective outer lipopolysaccharide membrane that reduces permeability to the SSZ molecule.

After 90 min of sonocatalytic treatment under optimal conditions, the toxicity of the solution decreased dramatically. Post-treatment GI% values fell to the range of 10–21% for all six strains. The largest absolute reductions were recorded for *H. influenzae* (from 95% to 10%) and *S. aureus* (from 98% to 11%). The residual GI% values (10–21%) reflect minor differences in bacterial susceptibility to the remaining low-molecular-weight organic acids and inorganic ions.

The substantial decline in antibacterial activity demonstrates that the sono-Fenton process successfully transformed SSZ into significantly less toxic intermediates. This detoxification is primarily attributed to the oxidative cleavage of the sulfonamide bond and the progressive degradation of the aromatic rings by hydroxyl radicals generated through the combined action of nZVI and ultrasound. Continuous cavitation maintains a steady supply of $\bullet\text{OH}$ radicals, which non-selectively attack the parent molecule and convert it into smaller organic acids and inorganic ions of lower biological activity [51].

The residual GI% values after treatment remain well below the commonly accepted ecotoxicological threshold of $\text{GI}\% < 25\%$ for safe discharge of treated wastewater into aquatic environments. The consistent and pronounced reduction across both Gram-positive and Gram-negative strains provides strong evidence that the US/nZVI/ H_2O_2 system not only achieves high degradation efficiency but also effectively detoxifies the treated effluent.

Conclusion

In this study, nanoscale zero-valent iron (nZVI) was successfully synthesized via a green method using oak leaf extract and applied as an efficient catalyst in a sono-Fenton system (US/nZVI/ H_2O_2) for the degradation of sulfasalazine (SSZ). Comprehensive characterization confirmed the formation of spherical to sub-spherical nanoparticles with a distinct core-shell structure (metallic Fe^0 core surrounded by an iron-oxide shell), an average particle size of approximately 32 nm, and a point of zero charge of 6.8. The presence of residual polyphenolic compounds on the particle surface contributed to colloidal stability and catalytic performance.

Under optimized conditions (pH 3.0, nZVI dosage 1.5 g L^{-1} , H_2O_2 concentration 1.5 mM, ultrasound power 150 W, and reaction time 90 min), the system achieved 95.8% degradation of SSZ. Kinetic analysis showed that the process followed pseudo-first-order kinetics with a rate constant of 0.0268 min^{-1} and a half-life of 25.9 min. A clear synergistic effect was observed among ultrasound, nZVI, and H_2O_2 , with the ternary system significantly outperforming individual and binary combinations.

TOC removal reached 86.7% after 120 min, indicating substantial mineralization of the parent compound and its intermediates. When applied to real pharmaceutical wastewater, the system achieved 78.4% SSZ removal, 55.6% COD reduction, 52.3% TOC removal, and 72.8% color removal, despite the presence of interfering matrix components. Toxicity assessment using six bacterial strains demonstrated a dramatic reduction in growth inhibition (from 89–98% to 10–21%), confirming effective detoxification of the treated effluent.

Overall, the US/nZVI/H₂O₂ process based on green-synthesized nZVI offers a highly efficient and environmentally friendly approach for the treatment of SSZ-contaminated water and pharmaceutical wastewater. The combination of high degradation and mineralization efficiencies together with significant toxicity reduction highlights the practical potential of this system for advanced wastewater treatment applications.

Statements and Declarations

Competing Interests:

The authors declare that they have no competing interests.

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Table and Figure captions

Table 1. Characteristics of the real pharmaceutical wastewater.

Table 2. Growth inhibition (GI%) of untreated and treated sulfasalazine against six bacterial strains.

Figure 1. SEM and TEM micrographs of fresh and spent nZVI: (A) SEM of fresh nZVI, (B) SEM of spent nZVI, (C) TEM of fresh nZVI, (D) TEM of spent nZVI.

Figure 2. XRD patterns of (A) fresh nZVI and (B) spent nZVI after sonocatalytic degradation of sulfasalazine.

Figure 3. (A) Zeta potential of green-synthesized nZVI as a function of pH; (B) EDS spectrum of green-synthesized nZVI.

Figure 4. Effect of operational parameters on SSZ degradation efficiency: (A) pH, (B) nZVI dosage, (C) H₂O₂ concentration, (D) ultrasound power, (E) initial SSZ concentration, and (F) reaction time.

Figure 5. Comparison of SSZ degradation efficiencies for individual, binary, and ternary systems under optimal conditions (synergistic study).

Figure 6. Degradation kinetics models under optimal conditions: (A) Pseudo-first-order kinetic plot; (B) Pseudo-second-order kinetic plot.

Figure 7. (A) Comparison of SSZ degradation and TOC removal versus time under optimal conditions; (B) Treatment efficiency of real pharmaceutical wastewater.

Figure 8. Bacterial growth inhibition (GI%) of untreated and treated SSZ solutions against six bacterial strains.

Table 1. Characteristics of the real pharmaceutical wastewater

Parameter	Value	Unit
pH	6.8 ± 0.3	–
COD	485 ± 25	mg L ⁻¹
TOC	195 ± 12	mg L ⁻¹
SSZ	45 ± 3	mg L ⁻¹
Color	1520	Pt-Co
TSS	85 ± 8	mg L ⁻¹
TDS	2150 ± 95	mg L ⁻¹
Electrical Conductivity (EC)	3.8 ± 0.2	mS cm ⁻¹
Dissolved Oxygen (DO)	2.5 ± 0.3	mg L ⁻¹

Table 2. Growth Inhibition (%) of Untreated and Treated Sulfasalazine Against Six Bacterial Strains.

Bacterial Strain	Untreated GI%	Treated GI%	Reduction in GI%
<i>Escherichia coli</i> ATCC 25922	93	16	77
<i>Pseudomonas aeruginosa</i> ATCC 9027	90	14	76
<i>Haemophilus influenzae</i> ATCC 49247	95	10	85
<i>Salmonella enterica</i> ATCC 19430	89	18	71

Bacterial Strain	Untreated GI%	Treated GI%	Reduction in GI%
<i>Staphylococcus aureus</i> ATCC 6538	98	11	87
<i>Streptococcus pneumoniae</i> ATCC 49619	91	21	70

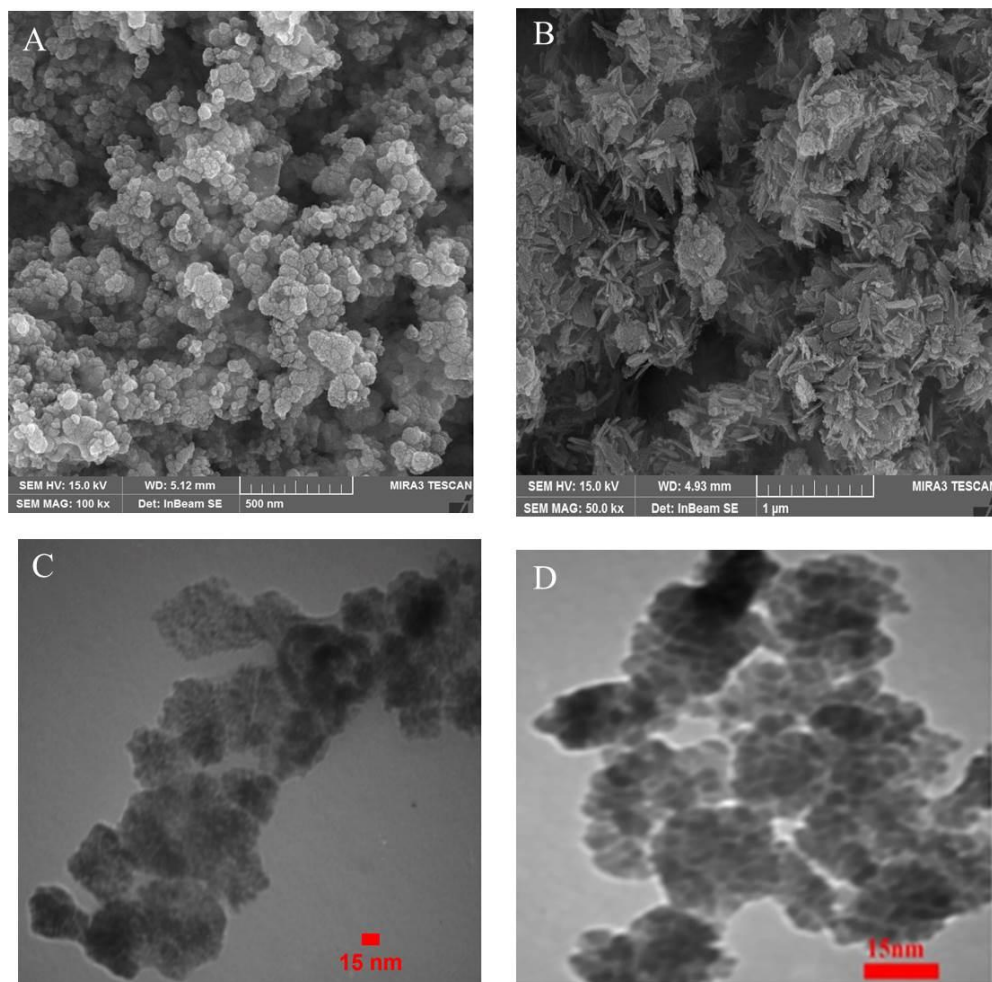


Figure 1. SEM and TEM micrographs of fresh and spent nZVI: (A) SEM of fresh nZVI, (B) SEM of spent nZVI, (C) TEM of fresh nZVI, (D) TEM of spent nZVI.

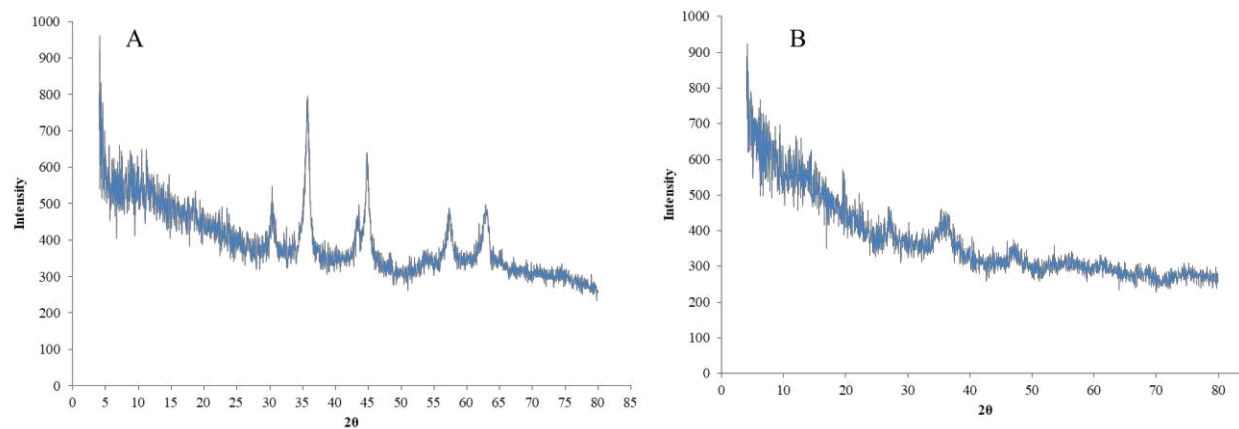


Figure 2. XRD patterns of (A) fresh nZVI and (B) spent nZVI after sonocatalytic degradation of sulfasalazine in the nZVI/H₂O₂/ultrasound system

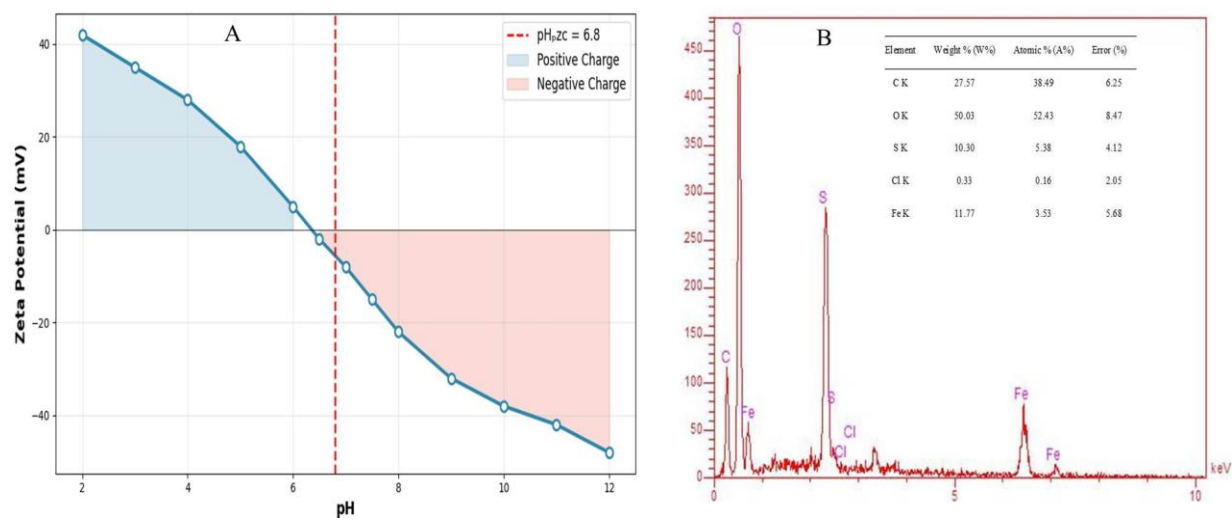


Figure 3. (A) Zeta potential of green-synthesized nZVI as a function of pH; (B) EDS spectrum of green-synthesized nZVI.

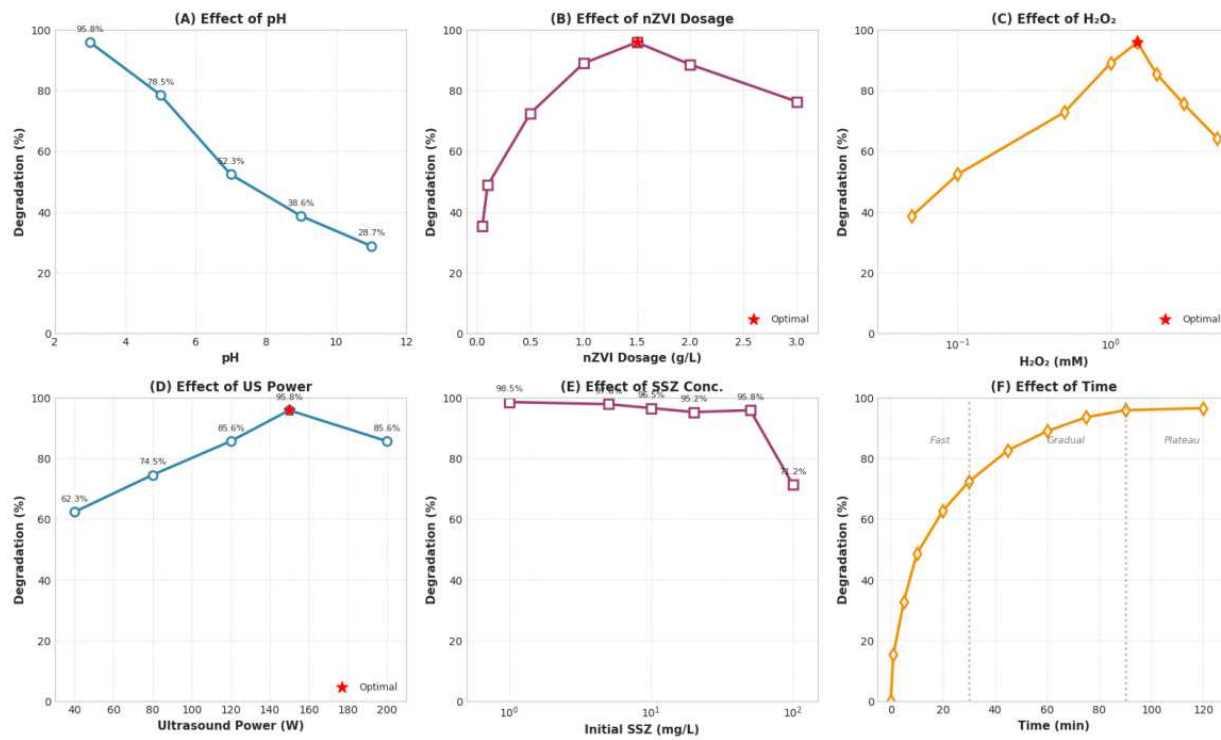


Figure 4. Effect of operational parameters on SSZ degradation efficiency.

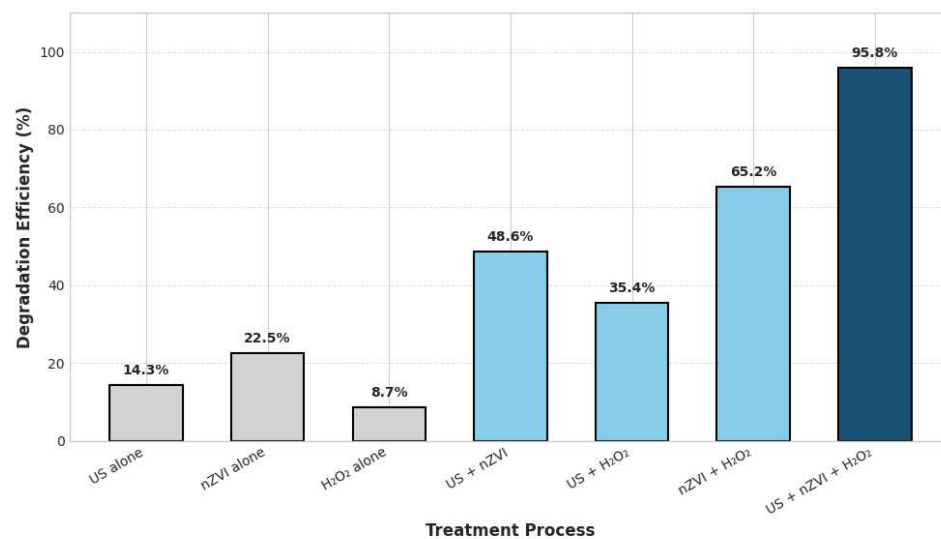


Figure 5. Synergistic study comparing degradation efficiencies of individual and combined processes

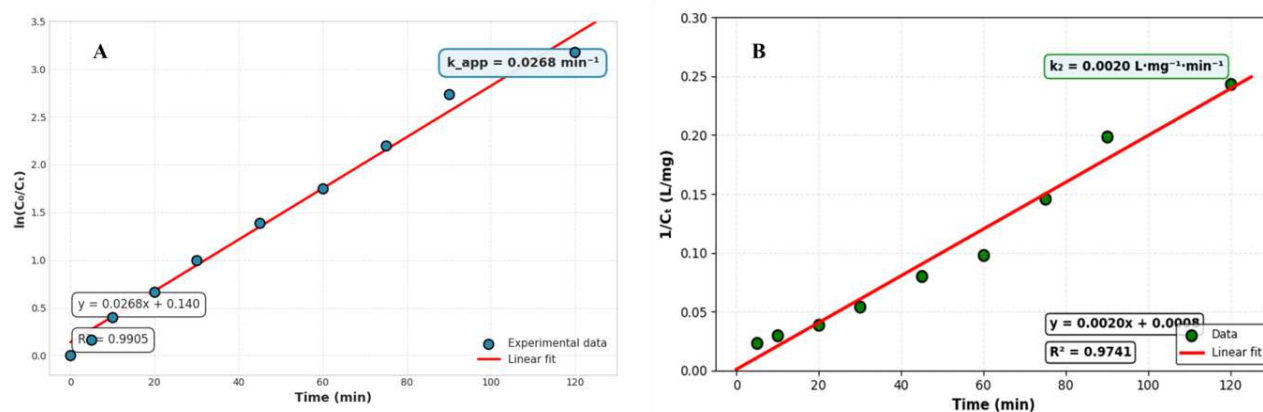


Figure 6. Degradation kinetics models: (A) Pseudo-first-order kinetic plot (B) Pseudo-second-order kinetic plot

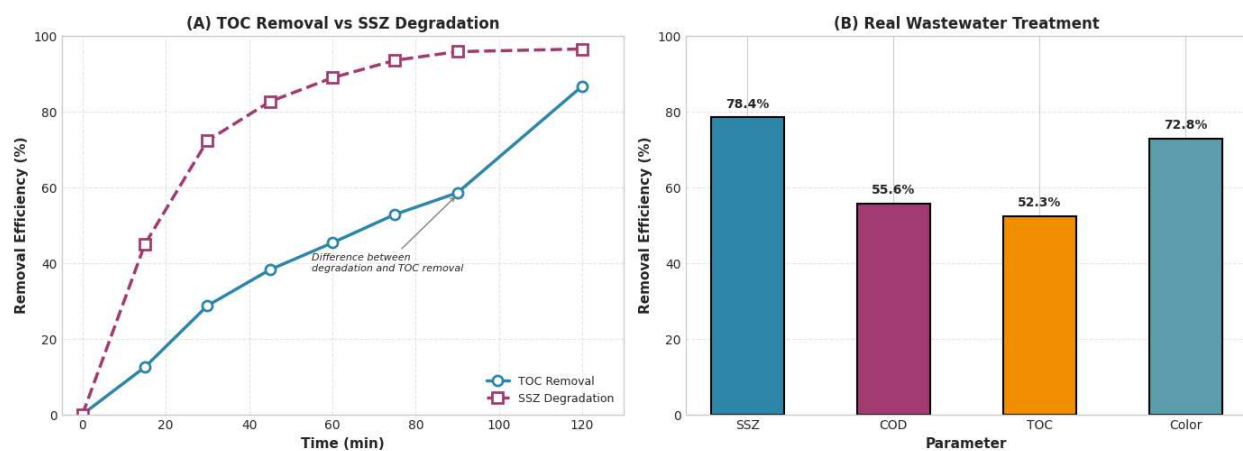


Figure 7. (A) Comparison of SSZ degradation and TOC removal versus time under optimal conditions; (B) Treatment efficiency of real pharmaceutical wastewater.

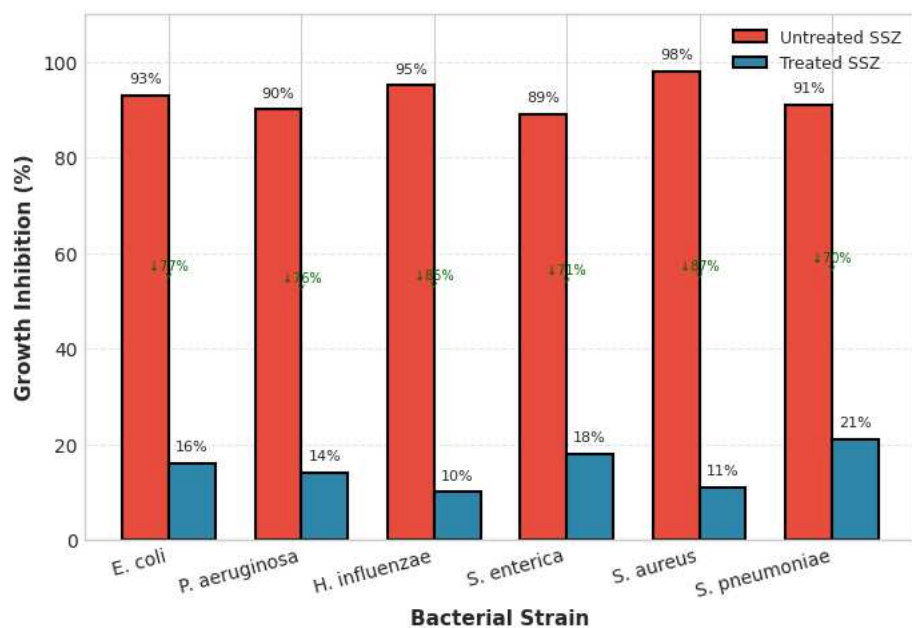


Figure 8. Bacterial growth inhibition (GI%) for untreated and treated SSZ against six bacterial strains