The Use of a Novel Graphitic Carbon Nitride/Cerium Dioxide (g-C\(_3\)N\(_4\)/CeO\(_2\)) Nanocomposites for the Ofloxacin Removal by Photocatalytic Degradation in Pharmaceutical Industry Wastewaters and the Evaluation of Microtox (\textit{Aliivibrio fischeri}) and \textit{Daphnia magna} Acute Toxicity Assays

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Abstract

In this study, a novel graphitic carbon nitride/cobalt molybdate (g-C\(_3\)N\(_4\)/CoMoO\(_4\)) nanocomposites (NCs) as a photocatalyst was examined during photocatalytic degradation process in the efficient removal of Ofloxacin (OFX) from pharmaceutical industry wastewater plant, Izmir, Turkey. Different pH values (3.0, 4.0, 6.0, 7.0, 9.0 and 11.0), increasing OFX concentrations (5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l), increasing g-C\(_3\)N\(_4\)/CeO\(_2\) NCs concentrations (1 mg/l, 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l and 10 mg/l), different g-C\(_3\)N\(_4\)/CeO\(_2\) NCs mass ratios (5/5, 6/4, 7/3, 8/2, 9/1, 1/9, 2/8, 3/7 and 4/6), increasing recycle times (1, 2, 3, 4, 5, 6 and 7) was operated during photocatalytic degradation process in the efficient removal of OFX in pharmaceutical industry wastewater. The characteristics of the synthesized nanoparticles (NPs) were assessed using X-Ray Diffractometry (XRD), Field Emission Scanning Electron Microscopy (FESEM), Energy-Dispersive X-ray (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), and Diffuse reflectance UV-Vis spectra (DRS) analyses, respectively. The acute toxicity assays were operated with Microtox (\textit{Aliivibrio fischeri} also called \textit{Vibrio fischeri}) and \textit{Daphnia magna} acute toxicity tests. The photocatalytic degradation mechanisms of g-C\(_3\)N\(_4\)/CeO\(_2\) NCs and the reaction kinetics of OFX were evaluated in pharmaceutical industry wastewater during photocatalytic degradation process. ANOVA statistical analysis was used for all experimental samples. The maximum 99% OFX removal efficiency was obtained during photocatalytic degradation process in pharmaceutical industry wastewater, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively. The maximum 99% OFX removal efficiency was found with photocatalytic degradation process in pharmaceutical industry wastewater, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min, at pH=6.0 and at 25°C, respectively. The maximum 99% OFX removal efficiency was measured to 8 mg/l g-C\(_3\)N\(_4\)/CeO\(_2\) NCs with photocatalytic degradation process in pharmaceutical industry wastewater, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min, at pH=6.0 and at 25°C, respectively. The maximum 99% OFX removal efficiency was measured at 2/8 wt g-C\(_3\)N\(_4\)/CeO\(_2\) NCs mass ratio at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min, at pH=6.0 and at 25°C, respectively. The maximum 99% OFX removal efficiency was measured in pharmaceutical industry wastewater during photocatalytic degradation process, after 1. recycle time, at 20 mg/l OFX, 8 mg/l g-C\(_3\)N\(_4\)/CeO\(_2\) NCs, at 2/8 wt g-C\(_3\)N\(_4\)/CeO\(_2\) NCs mass ratio, after 180 min, at pH=6.0 and at 25°C, respectively. 96.41% maximum Microtox (\textit{Aliivibrio fischeri}) acute toxicity removal yield was found in OFX=20 mg/l after 180 min photocatalytic degradation time and at 60°C. It was observed an inhibition effect of OFX=40 mg/l to Microtox with \textit{Vibrio fischeri} after 180 min and at 60°C. 92.38% maximum \textit{Daphnia magna} acute toxicity removal was obtained in OFX=20 mg/l after 180 min photocatalytic degradation time and at 60°C, respectively. It was observed an inhibition effect of OFX=40 mg/l to \textit{Daphnia magna} after 180 min and at 60°C. OFX concentrations > 20 mg/l decreased the acute toxicity removals by hindering the photocatalytic degradation process. Similarly, a significant contribution of increasing OFX concentrations to acute toxicity removal at 60°C after 180 min, was not observed. It can be concluded that the toxicity originating from the OFX is not significant and the real acute toxicity throughout photocatalytic degradation process was attributed to the pharmaceutical industry wastewater, to their metabolites and to the photocatalytic degradation process by-products. As a result, the a novel g-C\(_3\)N\(_4\)/CeO\(_2\) NCs photocatalyst during photocatalytic degradation process in pharmaceutical industry wastewater was stable in harsh environments such as acidic, alkaline, saline, and then was still effective process. When the amount of contaminant was increased, the a novel g-C\(_3\)N\(_4\)/CeO\(_2\) NCs photocatalysts during photocatalytic degradation process performance was still considerable. The synthesis and optimization of g-C\(_3\)N\(_4\)/CeO\(_2\) heterostructure photocatalyst provides insights into the effects of preparation conditions on the material’s characteristics and performance, as well as the application of the effectively designed photocatalyst in the removal of antibiotics, which can potentially be deployed for purifying wastewater, especially pharmaceutical wastewater. Finally, the combination of a simple, easy operation preparation process, excellent performance and cost effective, makes this a novel g-C\(_3\)N\(_4\)/CeO\(_2\) NCs a promising option during photocatalytic degradation process in pharmaceutical industry wastewater treatment.
Keywords: ANOVA statistical analysis, Antibiotics, Coronavirus Disease-2019 (COVID-19), Cost analysis, Diffuse reflectance UV-Vis spectra (DRS), Electrochemical filtration process, Energy-dispersive X-ray (EDX), Field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), Hydrothermal-calciation method, Hydroxyl (OH•) radicals, Microtox (Aliivibrio fischeri or Vibrio fischeri) and Daphnia magna acute toxicity tests, Nanoparticles (NPs), Novel graphitic carbon nitride/cerium dioxide nanocomposites (g-C3N4/CeO2 NCs), Ofloxacin (OFX), Pharmaceutical industry wastewater, Photocatalytic degradation mechanisms, Reaction kinetics, Sol–gel method, Transmission Electron Microscopy (TEM), Ultraviolet (UV), X-ray diffraction (XRD).

Introduction

Emerging contaminants (ECs), sometimes known as contaminants of emerging concern (CECs) can refer to a wide variety of artificial or naturally occurring chemicals or materials that are harmful to human health after long-term disclosure. ECs can be classified into several classes, including agricultural contaminants (pesticides and fertilizers), medicines and antidote drugs, industrial and consumer waste products, and personal care and household cleaning products [1,2]. Antibiotics are one of the ECs that have raised concerns in the previous two decades because they have been routinely and widely used in human and animal health care, resulting in widespread antibiotic residues discharged in surface, groundwater, and wastewater.

Antibiotics, which are widely utilized in medicine, poultry farming and food processing, have attracted considerable attention due to their abuse and their harmful effects on human health and the ecological environment. The misuse of antibiotics induces Deoxyribonucleic Acid (DNA) contamination and accelerates the generation of drug-resistant bacteria and super-bacteria thus, some diseases are more difficult to cure. A number of studies have revealed that the level of antibiotics in the soil, air and surface water, and even in potable water, is excessive in many areas, which will ultimately accumulate in the human body via drinking water and then damage the body’s nervous system, kidneys and blood system. Therefore, it is necessary to develop an efficient method to remove antibiotics present in pharmaceutical industry wastewater [3-13].

The uncontrolled, ever-growing accumulation of antibiotics and their residues in the environment is an acute modern problem. Their presence in water and soil is a potential hazard to the environment, humans, and other living beings. Many therapeutic agents are not completely metabolized, which leads to the penetration of active drug molecules into the biological environment, the emergence of new contamination sources, the wide spread of bacteria and microorganisms with multidrug resistance. Modern pharmaceutical wastewater facilities do not allow efficient removal of antibiotic residues from the environment, which leads to their accumulation in ecological systems. Global studies of river pollution with antibiotics have shown that 65% of surveyed rivers in 72 countries on 6 continents are contaminated with antibiotics [34-41].

The European Union’s Water Framework Directive enumerated certain antibiotics as priority contaminants. In some rivers, the concentrations were so high that they posed a real danger to both the ecosystem and human health. This matter, the development of effective approaches to the removal of antibiotics from the aquatic environment is of great importance [14-26].

The removal of antibiotics and their residues from water and wastewater prior to their final release into the environment is of particular concern. Modern purification methods can be roughly divided into the following three categories depending on the purification mechanism: biological treatment, chemical degradation, and physical removal. Each of these methods has its own advantages and disadvantages. For example, biological purification can remove most antibiotic residues, but the introduction of active organisms into the aquatic environment can upset the ecological balance. Various chemical approaches (ozonation, chlorination, and Fenton oxidation) cannot provide complete purification and, in some cases, lead to the death of beneficial microorganisms due to low selectivity. Photocatalysis is widely used in new environmental control strategies. However, this method has a number of key disadvantages, such as insufficient use of visible light, rapid annihilation of photogenerated carriers, and incomplete mineralization, which greatly limits its application [27-33].

Ofloxacin (OFX) is a quinolone antibiotic useful for the treatment of a number of bacterial infections. A quinolone antibiotic is a member of a large group of broad-spectrum bacteriocidals that share a bicyclic core structure related to the substance 4-quinolone. They are used in human and veterinary medicine to treat bacterial infections, as well as in animal husbandry, specifically poultry production. OFX is well-known for their antimicrobial and anti-inflammatory capabilities. OFX is used to treat pneumonia, skin and urinary tract infections. Severe acute respiratory syndrome (SARS)-CoV-2 (COVID-19) pandemic, which has killed and infected people in 216 countries/territories, has become the most significant pandemic of the century. OFX combined with other drugs, has been widely used to minimise COVID-19-induced inflammation in 2020.OFX is a typical fluoroquinolone antibiotic administered to both humans and animals, and after administration, approximately 78% of OFX is excreted. OFX pharmaceutical compounds enter water resources in various ways, such as human and animal excrections and inefficient industrial wastewater treatment. In the class of antibiotics, OFX is also recognised as highly refractory and persistent in aquatic water systems. As the biodegradation of OFX is difficult, sewage treatment plants (STPs) have a low removal rate, and the OFX concentrations in the STP effluents of Beijing, Hangzhou, and Vancouver have been determined to be between 6x10−7 and 1.405x10−5 mg/l [34-41].
Generally, the advanced oxidation processes (AOPs), such as the Fenton or Fenton-like reaction, ozonation or catalytic ozonation, photocatalytic oxidation, electrochemical oxidation, and ionizing radiation, have been widely used for antibiotics degradation in recent years. One of the most promising techniques applied for efficient degradation of antibiotics are Advanced Oxidation Processes (AOPs). Nowadays, particular attention is paid to photocatalytic reactions, in which highly oxidizing species responsible for mineralization of organic pollutants are formed in-situ in the reaction media by means of light and a photocatalyst. The photocatalytic activity is closely related to the physicochemical properties but also to the morphology and texture of the materials studied, for this reason the synthesis techniques are often of great importance. Photocatalysis, which occurs under exposure to UV light, is also a common method for the environmental pollutant elimination. The conventional photocatalysis utilizes mostly UV from sunlight, which accounts for only 4% of the solar energy. Therefore, through the introduction of catalysts, the utilization rate of sunlight can be effectively improved. To overcome the low-efficiency problem of the photocatalysis, the development of a more efficient catalyst system that would effectively improve the catalytic oxidation efficiency and overcome the existing limitations is important. The catalytic activity of the catalyst can be effectively improved by modifying its surface area, preparation method, and changing its properties and structures [42-57].

Numerous materials have been reported to have the potential and capacity to treat water or wastewater polluted with these antibiotics residue by applying the processes of adsorption and catalytic oxidation during the last few decades. The reported materials include mesoporous carbon beads, clay minerals, activated carbon, cellulose, and chitosan. As a result of engineering and science evolution, and in complement to the urgent need to increase the adsorption capability of antibiotic contaminants, more advanced materials such as carbon nanotube (CNT), nano-zero valent iron (nZVI), nanoporous carbons, porous graphene and graphene oxide (GO), to date have been analyzed and improved in their ability to remove these ECs from water [58-85].

Nanomaterials with a high specific surface area are a promising platform for the development and production of low-cost and highly efficient sorbents for various pollution molecules. For example, graphene-based nanomaterials were utilized to remove antibiotics, which are adsorbed on the material surfaces due to π-π-, electrostatic or hydrophobic interactions, as well as the formation of hydrogen bonds. Highly efficient antibiotic sorption was also observed when using highly porous, surface-active, and structurally stable silica-based materials, metal oxide NPs, and metal-organic frameworks. The photocatalysts, which mainly rely on the production of highly oxidizing species such as hydroxyl radical (OH·) and superoxide anion radical (O₂⁻), have been considered an effective approach for the degradation of antibiotics in water [86-103].

The two-dimensional (2D) g-C₃N₄ semiconductor has a wide range of applications in the environmental and energy fields because of its visible-light activity, unique physicochemical properties, excellent chemical stability and low-cost. Some important limitations of the photocatalytic activity of g-C₃N₄ are its low specific surface area, fast recombination of electrons and holes and poor visible light absorption. To improve the above problems, the construction of a heterojunction with a suitable band gap semiconductor (co-catalyst) has been shown to be a good strategy to improve the photocatalytic performance of g-C₃N₄, such as g-C₃N₄-based conventional type II heterojunctions, g-C₃N₄-based Z-scheme heterostructures, and g-C₃N₄-based p-n heterostructures, etc. The unique “Z” shape as the transport pathway of photo-generated charge carriers in Z-scheme photocatalytic systems is the most similar system to mimic natural photosynthesis in the many g-C₃N₄-based heterojunction photocatalysts. The construction of Z-scheme photocatalytic systems can promote visible light utilization and carrier separation, and maintain the strong reducibility and oxidizability of semiconductors. There are many studies on g-C₃N₄-based Z-scheme heterojunction photocatalysts, such as ZnO/g-C₃N₄, WO₃/g-C₃N₄, g-C₃N₄/ZnS, g-C₃N₄/Fe₃O₄, g-C₃N₄/graphene/NiFe₂O₄, NiCo/ZnO/g-C₃N₄ and Bi₂ZrO₃/g-C₃N₄/Ag₃PO₄, respectively. g-C₃N₄-based Z-scheme heterojunction photocatalysts have been made to improve the photocatalytic activity by combining with other semiconductor materials. Therefore, there are some problems with the single photocatalytic method, such as low adsorption ability, limited active sites and low removal efficiency. The integration of the adsorption and photocatalytic degradation of various organic pollutants is considered as a suitable and promising technology. On the other hand, it is still essential to fabricate photocatalysts with superior adsorption and degradation efficiencies [104-121].

g-C₃N₄ has been gaining great attention as a potential photocatalyst due to its stability and safety characteristics, as well as the fact that it can be facilely synthesized from low-cost raw materials. The low bandgap (~2.7 eV) can drive photo-oxidation reactions even under visible light. However, the pure g-C₃N₄ has some drawbacks such as its low redox potential and high rate of recombination between photo-induced electrons and holes, which dramatically limits its photocatalytic efficiency. Several strategies have been investigated, including modification of the material's size and structure, nonmetal and metal doping, and coupling with other photocatalysts. For example, Liu et al. improved bulk g-C₃N₄'s performance in terms of Rhodamine B degradation from 30% to 100% by synthesizing mesoporous g-C₃N₄ nanorods through the nano-confined thermal condensation method. Dai et al. doped g-C₃N₄ with Cu through a thermal polymerization route and acquired a degradation rate of 90.5% with norfloxacin antibiotic. Nithya and Ayappan, synthesized hybridized g-C₃N₄/ZnB₄O₁₁ for reduction of 4-nitrophenol and reached an optimal removal efficiency of 79%. Among all, the construction of heterostructure photocatalysts by coupling g-C₃N₄ with other semiconductors seems to be an effective strategy to prevent electron and hole recombination, hence improving photocatalytic efficiency for contaminant treatment [122-131].

CeO₂ (Ceria or Cerium(IV) oxide) is a versatile, inert, and physically and chemically stable material with multiple and diverse applications. Due to its hardness (Mohs scale 7), it was initially used as an abrasive material, but today it is used (alone or in binary or complex mixtures) in the field of heterogeneous catalysis (oxidation of hydrocarbons) or in the field of sensors, energy, and fuels such as solid oxide fuel cells, but also in water-splitting processes or photocatalysis. CeO₂ applications in the dermato-cosmetics industry and in the

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biomedical field (antibacterial effect) should also be mentioned here. CeO\textsubscript{2} is also possible to combine two or more properties, for example, the infrared filtering properties with the photocatalytic ones, to optimize practical applications. CeO\textsubscript{2} is semiconductor photocatalyst with various applications and similar properties to TiO\textsubscript{2}. However, its band gap is in the wide range of 2.6 to 3.4 eV, depending on the preparation method. Furthermore, CeO\textsubscript{2} exhibits promising photocatalytic activity. Nonetheless, the position of CB and VB limits its application as an efficient photocatalyst utilizing solar energy, even though CeO\textsubscript{2} can absorb a larger fraction of the solar spectrum than TiO\textsubscript{2}. The photocatalytic and photoelectrocatalytic activity of CeO\textsubscript{2} in wastewater treatment can be improved by various modification techniques, including changes in morphology, doping with metal cation dopants and non-metal dopants, coupling with other semiconductors, combining it with carbon supporting materials, etc. The main properties that make CeO\textsubscript{2} significant as a photocatalyst and photoelectrode material applied in the degradation of various pollutants result from its high band gap energy, high refractive index, high optical transparency in the visible region, high oxygen storage capacity, and chemical reactivity. The other properties of CeO\textsubscript{2} which should be mentioned include its high thermal stability, high hardness, oxygen ion conductivity, special redox features, and easy conversion between Ce\textsuperscript{3+} and Ce\textsuperscript{4+} oxidation states \[132-156]\.

The conduction band (CB) of g-C\textsubscript{3}N\textsubscript{4} is more negative than that of CeO\textsubscript{2} (-1.24 eV and -0.44, respectively), while CeO\textsubscript{2} possesses a relatively positive valance band (VB) (2.56 eV) compared to the conduction band of g-C\textsubscript{3}N\textsubscript{4}. Would theoretically facilitate the electron conduction band of CeO\textsubscript{2}, would significantly increase, leading to the decomposition of organic compounds by O\textsuperscript{2-} and OH\textsuperscript{-} reactive species.

In this study, a novel g-C\textsubscript{3}N\textsubscript{4}/CeO\textsubscript{2} NCs as a photocatalyst was examined during photocatalytic degradation process in the efficient removal of OFX from pharmaceutical industry wastewater plant, Izmir, Turkey. Different pH values (3.0, 4.0, 6.0, 7.0, 9.0 and 11.0), increasing OFX concentrations (5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l), increasing g-C\textsubscript{3}N\textsubscript{4}/CeO\textsubscript{2} NCs concentrations (1 mg/l, 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l, and 10 mg/l), different g-C\textsubscript{3}N\textsubscript{4}/CeO\textsubscript{2} NCs mass ratios (2/5, 6/4, 7/3, 8/2, 9/1, 1/9, 2/8, 3/7, 4/6), increasing recycle times (1, 2, 3, 4, 5, 6 and 7) was operated during photocatalytic degradation process in the efficient removal of OFX in pharmaceutical industry wastewater. The characteristics of the synthesized NPs were XRD, FESEM, EDX, FTIR, TEM and DRX analyses, respectively. The acute toxicity assays were operated with Microtox (Aliivibrio fischeri and Daphnia magna acute toxicity tests. The photocatalytic degradation mechanisms of g-C\textsubscript{3}N\textsubscript{4}/CeO\textsubscript{2} NCs and the reaction kinetics of OFX were evaluated in pharmaceutical industry wastewater during photocatalytic degradation process. ANOVA statistical analysis was used for all experimental samples.

Materials and Methods

Characterization of Pharmaceutical Industry Wastewater

Characterization of the biological aerobic activated sludge process from a pharmaceutical industry wastewater plant, Izmir, Turkey was performed. The results are given as the mean value of triplicate samplings (Table 1).

![Table 1: Characterization of Pharmaceutical Industry Wastewater.](image-url)
Preparation of Graphitic Carbon Nitride (g-C₃N₄) Nanoparticles

g-C₃N₄ was prepared by calcination of melamine (C₆H₆N₆) in a crucible with a lid at 550°C for 4 h. The obtained yellow powder was ground in an agate mortar after being cooled down to 25°C room temperature.

Preparation of Cerium Dioxide (CeO₂) Nanoparticles

CeO₂ NPs were prepared by sol–gel method. Nano-sized CeO₂ was also prepared by the sol–gel procedure using Cerium nitrate hexahydrate [Ce(NO₃)₃·6H₂O] and 20 ml of Triethanolamine (C₆H₁₅NO₃). Then, they were mixed together by a magnetic stirrer on a hot plate to insure that the cerium salt was dissolved in C₆H₁₅NO₃. After that the solution was heated up to 90°C until the clear dark brown homogenous solution, sol, was observed. To prepare black colloidal solution (gel), it was kept in a digital furnace at 270°C for 2 h. As gel was produced, it was cooled to 25°C room temperature. In order to form the expected precipitate, the volume of the gel solution was adjusted to 100 ml by adding ethanol (C₂H₆O). Then, synthesized precipitate was separated by centrifugation and washed by deionized water and C₂H₆O. Finally, the produced CeO₂ NPs was dried at 90°C and calcinated.

Preparation of A Novel Graphitic Carbon Nitride/Cerium Dioxide (g-C₃N₄/CeO₂) Nanocomposites

The g-C₃N₄/CeO₂ NCs was synthesized by the hydrothermal-calcination method. Firstly, 1 gram g-C₃N₄ NPs was added into distilled water and magnetically stirred for 30 min. Then, the portions of prepared g-C₃N₄ NPs were added to the mixtures to obtain the mass ratios of g-C₃N₄ to CeO₂ of 5/5, 6/4, 7/3, 8/2, 9/1, 1/9, 2/8, 3/7 and 4/6, respectively, and kept being stirred for another 1 h. The final mixtures were transferred into a 100 ml autoclave and reacted at 180°C for different hydrothermal (HT) times of 2 h, 4 h and 6 h. The final samples were centrifuged and washed with distilled water and C₂H₆O for 2 times. Then, the samples were dried, and finally, the dried products were heated in a Muffle furnace at different calcination temperatures of 300°C, 400°C and 500°C for 4 h to get the target composites. The synthesis conditions and the corresponding sample names were summarized at Table 2.

Characterization

X-Ray Diffraction Analysis

Powder XRD patterns were recorded on a Shimadzu XRD-7000, Japan diffractometer using Cu Ka radiation (λ = 1.5418 Å, 40 kV, 40 mA) at a scanning speed of 1°/min in the 10–80° 2θ range. Raman spectrum was collected with a Horiba Jobin Yvon-Labram HR UV-Visible NIR (200-1600 nm) Raman microscope spectrometer, using a laser with the wavelength of 512 nm. The spectrum was collected from 10 scans at a resolution of 2 /cm. The zeta potential was measured with a SurPASS Electrokinektic Analyzer (Austria) with a clamping cell at 300 mbar.

Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-Ray (EDX) Spectroscopy Analysis

The morphological features and structure of the synthesized catalyst were investigated by FESEM (FESEM, Hitachi S-4700), equipped with an EDX spectrometry device (TESCAN Co., Model III MIRA) to investigate the composition of the elements present in the synthesized catalyst.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectra of samples was recorded using the FT-NIR spectroscope (RAYLEIGH, WQF-510).

Transmission Electron Microscopy (TEM) Analysis

The structure of the samples were analysed TEM analysis. TEM analysis was recorded in a JEOL JEM 2100F, Japan under 200 kV accelerating voltage. Samples were prepared by applying one drop of

Table 2: The optimization parameters of g-C₃N₄/CeO₂ NCs samples.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mass Ratios of g-C₃N₄/CeO₂ NCs</th>
<th>Calcination Temperature (°C) in 240 min</th>
<th>Hydrothermal Time (min) at 180°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-2h-Cal500</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-2h-Cal400</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-2h-Cal300</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-4h-Cal500</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-4h-Cal400</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
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<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-6h-Cal500</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-6h-Cal400</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>HT-6h-Cal300</td>
<td>8/2</td>
<td>500°C</td>
<td>360</td>
</tr>
<tr>
<td>5/5 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>5/5</td>
<td>500°C</td>
</tr>
<tr>
<td>6/4 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>6/4</td>
<td>500°C</td>
</tr>
<tr>
<td>7/3 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>7/3</td>
<td>500°C</td>
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<tr>
<td>8/2 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>8/2</td>
<td>500°C</td>
</tr>
<tr>
<td>9/1 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>9/1</td>
<td>500°C</td>
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<tr>
<td>1/9 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>1/9</td>
<td>500°C</td>
</tr>
<tr>
<td>2/8 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>2/8</td>
<td>500°C</td>
</tr>
<tr>
<td>3/7 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>3/7</td>
<td>500°C</td>
</tr>
<tr>
<td>4/6 wt. g-C₃N₄/CeO₂</td>
<td></td>
<td>4/6</td>
<td>500°C</td>
</tr>
</tbody>
</table>
the suspended material in ethanol onto a carbon-coated copper TEM grid, and allowing them to dry at 25°C room temperature.

**Diffuse Reflectance UV-Vis Spectra (DRS) Analysis**

DRS Analysis in the range of 200–800 nm were recorded on a Cary 5000 UV-Vis Spectrophotometer from Varian. DRS was used to monitor the OFX antibiotic concentration in experimental samples.

**Analytical Procedures**

Chemical oxygen demand-total (COD\text{total}), chemical oxygen demand-dissolved (COD\text{dissolved}), total phosphorus (Total-P), phosphate phosphorus (PO\text{4}^{3-}), total nitrogen (Total-N), ammonium nitrogen (NH\text{4}^{+}), nitrate nitrogen (NO\text{3}^{-}), nitrite nitrogen (NO\text{2}^{-}), biological oxygen demand 5-days (BOD \text{5}), pH, Temperature (°C), total suspended solids (TSS), volatile suspended solids (TVSS), total organic carbon (TOC), Oil, Chloride (Cl\text{-}), total phenol, total volatiles acids (TVA), dissolved organic carbon (DOC), total alkalinity, turbidity, dissolved solid (TDS), color, sulfide (SO\text{2}^{−}), sulfate (SO\text{4}^{2−}), bicarbonate (HCO\text{3}^{−}), salinity, cobalt (Co\text{+3}), lead (Pb\text{+2}), potassium (K\text{+}), iron (Fe\text{+2}), chromium (Cr\text{+2}), Mercury (Hg\text{+2}), and zinc (Zn\text{+2}) were measured according to the Standard Methods (2017) 5220B, 5220D, 4500-P, 4500-PO\text{4}^{3-}, 4500-N, 4500-NH\text{4}^{+}, 4500-NO\text{3}^{−}, 4500-NO\text{2}^{-}, 5210B, 4500-H\text{2}, 2320, 2540D, 2540E, 5310, 5520, 4500-Cl\text{-}, 5530, 5560B, 5310B, 2320, 2130, 2540E, 2120, 4500-SO\text{4}^{2−}, 4500-SO\text{3}^{−}, 5320, 2520, 3500-Co\text{+3}, 3500-Pb\text{+2}, 3500-K\text{+}, 3500-Fe\text{+2}, 3500-Cr\text{+2}, 3500-Hg\text{+2}, 3500-Zn\text{+2}, respectively [158].

Total-N, NH\text{4}^{+}, NO\text{3}^{-}, NO\text{2}^{-}, Total-P, PO\text{4}^{3-}, P, total phenol, Co\text{+3}, Pb\text{+2}, K\text{+}, Fe\text{+2}, Cr\text{+2}, Hg\text{+2}, Zn\text{+2}, SO\text{3}^{2−}, SO\text{4}^{2−}, and SO\text{2}^{−} were measured with cell test spectroquant kits (Merck, Germany) at a spectroquant NOVA 60 (Merck, Germany) spectrophotometer (2003).

The measurement of color was carried out following the methods described by Oltthof and Eckenfelder [159] and Eckenfelder [160]. According these methods, the color content was determined by measuring the absorbance at three wavelengths (445 nm, 540 nm and 660 nm), and taking the sum of the absorbances at these wavelengths. In order to identify the color in pharmaceutical industry wastewater (25 ml) was acidified at pH=2.0 with a few drops of 6 N HCl and extracted three times with 25 ml of ethyl acetate. The pooled organic phases were dehydrated on sodium sulphate, filtered and dried under vacuum. The residue was syllilated with bis(trimethylsilyl) trifluoroacetamide (BSTFA) in dimethylformamide and analyzed by gas chromatography–mass spectrometry (GC-MS) and gas chromatography (GC) (Agilent Technology model 6890N) equipped with a mass selective detector (Agilent 5973 inert MSD). Mass spectra were recorded using a VGTS 250 spectrometer equipped with a capillary SE 52 column (HP5-MS 30 m, 0.25 mm ID, 0.25 μm) at 220°C with an isothermal program for 10 min. The initial oven temperature was kept at 50°C for 1 min, then raised to 220°C at 25°C/min and from 200 to 300°C at 8°C/min, and was then maintained for 5.5 min. High purity He (g) was used as the carrier gas at constant flow mode (1.5 ml/min, 45 cm/s linear velocity).

The total phenol was monitored as shown: 40 ml of pharmaceutical industry wastewater was acidified to pH=2.0 by the addition of concentrated HCl. Total phenol was then extracted with ethyl acetate. The organic phase was concentrated at 40°C to about 1 ml and silylized by the addition of N,O-bis(trimethylsilyl) acetamide (BSA). The resulting trimethylsilyl derivatives were analysed by GC-MS (Hewlett-Packard 6980/HP5973MSD).

Methyl tertiary butyl ether (MTBE) was used to extract oil from the water and NPs. GC-MS analysis was performed on an Agilent gas chromatography (GC) system. Oil concentration was measured using a UV–vis spectroscopy fluorescence spectroscopy and a GC–MS (Hewlett-Packard 6980/HP5973MSD). UV–vis absorbance was measured on a UV–vis spectrophotometer and oil concentration was calculated using a calibration plot which was obtained with known oil concentration samples.

**Acute Toxicity Assays**

**Microtox Acute Toxicity Test**

Toxicity to the bioluminescent organism *Aliivibrio fischeri* (also called *Vibrio fischeri* or *V. fischeri*) was assayed using the Microtox measuring system according to DIN 38412L34, L34L, (EPS 1/ RM/24 1992). Microtox testing was performed according to the standard procedure recommended by the manufacturer [161]. A specific strain of the marine bacterium, *V. fischeri-Microtox* LCK 491 kit was used for the Microtox acute toxicity assay. Dr. LANGE LUMIX-mini type luminometer was used for the microtox toxicity assay [162].

**Daphnia magna Acute Toxicity Test**

To test toxicity, 24-h born *Daphnia magna* were used as described in Standard Methods sections 8711A, 8711B, 8711C, 8711D and 8711E, respectively [163]. After preparing the test solution, experiments were carried out using 5 or 10 *Daphnia magna* introduced into the test vessels. These vessels had 100 ml of effective volume at 7.0– 8.0 pH, providing a minimum dissolved oxygen (DO) concentration of 6 mg/l at an ambient temperature of 20–25°C. Young *Daphnia magna* were used in the test (≤24 h old); 24–48 h exposure is generally accepted as standard for a *Daphnia magna* acute toxicity test. The results were expressed as mortality percentage of the *Daphnia magna*. Immobile animals were reported as dead *Daphnia magna*.

**Statistical Analysis**

ANOVA analysis of variance between experimental data was performed to detect F and P values. The ANOVA test was used to test the differences between dependent and independent groups. Comparison between the actual variation of the experimental data averages and standard deviation is expressed in terms of F ratio. F is equal (found variation of the date averages/expected variation of the date averages) and P indicates the number of degrees of freedom. Regression analysis was applied to the experimental data in order to determine the regression coefficient R\text{2}. [165]. The aforementioned test was performed using Microsoft Excel Program.

All experiments were carried out three times and the results are given as the means of triplicate samplings. The data relevant to the individual pollutant parameters are given as the mean with standard deviation (SD) values.
Results and Discussions

A Novel g-C₃N₄/CeO₂ NCs Characteristics

The Results of X-Ray Diffraction (XRD) Analysis

The results of XRD analysis was observed to pure g-C₃N₄ NPs, pure CeO₂ NPs and g-C₃N₄/CeO₂ NCs, respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 1). The characterization peaks were observed at 2θ values of 14.21°, 20.12° and 28.24°, respectively, implying pure g-C₃N₄ NPs in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 1a). The characterization peaks were obtained at 2θ values of 29.41°, 34.22°, 48.45°, 57.62°, 59.27°, 70.18°, 77.17° and 79.31°, respectively, implying pure CeO₂ NPs in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 1b). The characterization peaks were found at 2θ values of 13.20°, 28.72°, 33.67°, 48.15°, 58.39°, 60.16°, 71.17°, 75.35° and 79.53°, respectively, and which can also be indexed as (100), (002), (200), (220), (311), (222), (400), (331) and (420), respectively, implying g-C₃N₄/CeO₂ NCs in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 1c).

The Results of Diffuse Reflectance UV-Vis Spectra (DRS) Analysis

The absorption spectra of OFX was observed in DRS Analysis (Figure 2). First, the absorption spectra of OFX were obtained at a maximum concentration of 40 mg/l in the wavelength range from 250 nm to 800 nm using diffuse reflectance UV-Vis spectra (Figure 2). Absorption peaks were observed at wavelengths of 400 nm for pure g-C₃N₄ NPs (black pattern) (Figure 2a), 310 nm for pure CeO₂ NPs (green pattern) (Figure 2b), and 340 nm for g-C₃N₄/CoMoO₄ NCs (blue pattern) (Figure 2c), respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.

The Results of Field Emission Scanning Electron Microscopy (FESEM) Analysis

The morphological features of pure g-C₃N₄ NPs, pure CeO₂ NPs and g-C₃N₄/CeO₂ NCs were characterized through FE-SEM images (Figure 3). The FESEM images of pure g-C₃N₄ NPs were obtained in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 3a). The FESEM images of pure CeO₂ NPs were observed in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 3b). The FESEM images of g-C₃N₄/CeO₂ NCs were characterized in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 3c).

The Results of Energy Dispersive X-Ray (EDX) Spectroscopy Analysis

The EDX analysis was also performed to investigate the composition of g-C₃N₄/CeO₂ NCs (Figure 4), respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.

The Results of Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrum of pure g-C₃N₄ NPs (black spectrum), pure CeO₂ NPs (blue spectrum) and g-C₃N₄/CeO₂ NCs (red spectrum), respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 5). The main peaks of FTIR spectrum for pure g-C₃N₄ NPs (black spectrum) was observed at 1645 1/cm, 1564 1/cm, 1411 1/cm, 1321 1/cm, 1240 1/cm and 807 1/cm wavenumber, respectively (Figure 5a). The main peaks of FTIR spectrum for pure CeO₂ NPs (blue spectrum) was determined at 462 1/cm wavenumber, respectively (Figure 5b). The main peaks of FTIR spectrum for g-C₃N₄/CeO₂ NCs (red spectrum) was determined at 462 1/cm wavenumber, respectively (Figure 5c).

Figure 1: The XRD patterns of (a) pure g-C₃N₄ NPs (black pattern), (b) pure CeO₂ NPs (blue pattern) and (c) g-C₃N₄/CeO₂ NCs (red pattern), respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.

Figure 2: The DRS patterns of (a) pure g-C₃N₄ NPs (black pattern) (b) pure CeO₂ NPs (green pattern) and (c) g-C₃N₄/CeO₂ NCs (blue pattern), respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.
The Use of a Novel Graphitic Carbon Nitride/Cerium Dioxide ($g$-$C_3N_4$/CeO$_2$) Nanocomposites for the Ofloxacin Removal by Photocatalytic Degradation in Pharmaceutical Industry Wastewaters and the Evaluation of Microtox ($Aliivibrio fischeri$) and $Daphnia magna$ Acute Toxicity Assays

The Results of Transmission Electron Microscopy (TEM) Analysis

The TEM images of $g$-$C_3N_4$/CeO$_2$ NCs was observed in micromorphological structure level in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal (Figure 6).

The Reaction Kinetics of OFX Antibiotic

The reaction kinetics OFX were investigated using the Langmuir–Hinshelwood first-order kinetic model, expressed by Eddy et al. [166], as following Equation (1):

\[
\frac{dr}{dt} = -kc = kC
\]

where; $r_i$: denotes the initial photocatalytic degradation reaction rate ($\text{mg} / \text{L.min}$), and $k$: denotes the rate constant of a first-order

Figure 3: FESEM images of (a) pure $g$-$C_3N_4$ NPs, (b) pure CeO$_2$ NPs and (c) $g$-$C_3N_4$/CeO$_2$ NCs, respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.

Figure 4: EDX spectrum of $g$-$C_3N_4$/CeO$_2$ NCs, respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.

Figure 5: FTIR spectrum of (a) pure $g$-$C_3N_4$ NPs (black spectrum), (b) pure CeO$_2$ NPs (blue spectrum) and (c) $g$-$C_3N_4$/CeO$_2$ NCs (red spectrum), respectively, in pharmaceutical industry wastewater with photocatalytic degradation process for OFX antibiotic removal.
reaction. At the beginning of the reaction, \( t = 0 \), \( C_0 = C_f \), the equation can be obtained after integration as following Equation (2):

\[
\ln \frac{C_f}{C_0} = -kt \quad (2)
\]

where: \( C_0 \) and \( C_f \) are the initial and final concentration (mg/l) of OFX; the solution at \( t \) (min) and \( k \) (1/min) are the rate constant.

The correlation coefficients had \( R^2 \) values greater than 0.9, as a result, the first-order kinetic model fit the experimental data well. The first-order rate constants (\( k \)) were determined from the slope of the linear plots.

**Photocatalytic Degradation Mechanisms**

The photocatalytic performance of the catalyst in the degradation of OFX is determined by photons. The degradation mechanism of OFX by hydroxyl radicals (OH*) radicals concerning \( g-C_3N_4/\text{CeO}_2 \) NCSs as following equations (Equation 3, Equation 4, Equation 5, Equation 6, Equation 7, Equation 8, Equation 9 and Equation 10):

\[
g - C_3N_4/\text{CeO}_2 + h\nu \rightarrow g - C_3N_4/\text{CeO}_2 (h^+ + e^-)/\text{CeO}_2 (h^+ + e^-) - g - C_3N_4/\text{CeO}_2 (e^- + e^-) \quad (3)
\]

\[
e^- + O_2 \rightarrow O_2^- \quad (5)
\]

\[
h^+ + \text{H}_2\text{O} \rightarrow \text{OH}^* + h^+ \quad (6)
\]

\[
\text{CeO}_2 (h^+) + \text{H}_2\text{O} \rightarrow \text{OH}^* + h^+ + \text{TiO}_2 \quad (7)
\]

\[
\text{OH}^- + \text{CeO}_2 (h^+) \rightarrow \text{OH}^* + \text{TiO}_2 \quad (8)
\]

\[
O_2^- + e^- \rightarrow O_2^* \quad (9)
\]

\[
\text{OFX} + O_2^*/\text{OH}^* + \text{CO}_2 + \text{H}_2\text{O} + \text{Degradation Products} \quad (10)
\]

\( g-C_3N_4/\text{CeO}_2 \) NCSs absorbs photons with energies greater than the photocatalyst bandgap. As a result, the electron in the valence band (VB) jumps to the conduction band (CB), leaving a hole in the CB. The electrons present in the CB and VB will react with oxygen (O\(_2\)) and water (H\(_2\)O) molecules which are absorbed by the photocatalyst and lead to the formation of OH* radicals which react with OFX. OH* radicals are produced when the photocatalyst surface is illuminated with photons, and OH* radicals are strong oxidising species, with an oxidation potential of approximately 2.8 V [as opposed to Normal hydrogen electrode (NHE)], which may increase total pollutant mineralisation. Normally, the higher the rate of formation of OH* radicals, the greater the separation efficiency of electron-hole pairs. In this way, there is a correlation between the increased photocatalytic activity and the rate of formation of OH* radicals. The OH* radicals generation of \( g-C_3N_4/\text{CeO}_2 \) NCs was extremely high, indicating that the sample has a high electron and hole separation rate.

\( \text{CeO}_2 \) composites with \( g-C_3N_4 \) are also promising photocatalytic materials with a lower band gap energy [167-169] and significantly higher photocatalytic efficiency in degradation processes [170,171]. Considering the position of CB and VB in \( \text{CeO}_2 \) and \( g-C_3N_4 \), the higher photocatalytic efficiency can be attributed to the transfer of photoexcited electrons and holes between \( \text{CeO}_2 \) and \( g-C_3N_4 \), which suppresses the recombination of photogenerated h\(^+\)/e\(^-\) pairs. During irradiation, photogenerated electrons on CB in \( g-C_3N_4 \) are transferred to CB in \( \text{CeO}_2 \) and react with O\(_2^-\), while photogenerated holes on VB in \( \text{CeO}_2 \) are transferred to VB in \( g-C_3N_4 \) and react with H\(_2\)O according to the following reactions [172]:

The superoxide and hydroxyl radicals formed in the above-presented reactions take part in the degradation of pollutants. In the case of \( \text{CeO}_2 \) composites with \( g-C_3N_4 \), two problems have still not been resolved. The first one is related to the lower rates of TOC or COD decrease in wastewater in comparison with the degradation rate of pollutants [173]. The second one is attributed to the immobilization of a composite photocatalyst, which could eliminate the post-treatment process of photocatalyst removal from the wastewater.

**Effect of Increasing pH values for OFX Removal in Pharmaceutical Industry Wastewater during Photocatalytic Degradation Process**

Increasing pH values (pH=3.0, pH=4.0, pH=6.0, pH=7.0, pH=9.0 and pH=11.0, respectively) was examined during photocatalytic degradation process in pharmaceutical industry wastewater for OFX removal (Figure 7). 67.2%, 85.7%, 96.4%, 56.5% and 44.8% OFX removal efficiencies was measured at pH=3.0, pH=4.0, pH=6.0, pH=7.0 and pH=11.0, respectively, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at 25°C (Figure 7). The maximum 99% OFX removal efficiency was obtained during photocatalytic degradation process in pharmaceutical industry wastewater, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 7).

**Effect of Increasing OFX Concentrations for OFX Removal in Pharmaceutical Industry Wastewater during Photocatalytic Degradation Process**

Increasing OFX concentrations (5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l) were operated at 300 W UV-vis irradiation power, after 180 min photocatalytic degradation time, at pH=6.0, at 25°C, respectively (Figure 8). 85.3%, 94.1% and 77.2% OFX removal efficiencies were obtained to 5 mg/l, 10 mg/l and 40 mg/l OFX concentrations, respectively, at pH=6.0 and at 25°C (Figure 8). The maximum 99% OFX removal efficiency was found with photocatalytic degradation.
Delia Teresa Sponza (2023) The Use of a Novel Graphitic Carbon Nitride/Cerium Dioxide (g-C$_3$N$_4$/CeO$_2$) Nanocomposites for the Ofloxacin Removal by Photocatalytic Degradation in Pharmaceutical Industry Wastewaters and the Evaluation of Microtox (Aliivibrio fischeri) and Daphnia magna Acute Toxicity Assays

process in pharmaceutical industry wastewater, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 8).

The percentage decrease (8%) in the concentration of OFX during the studies under the dark conditions was due to the contaminant adsorption onto the catalyst surface [174]. The formation of contaminant monolayer on the surface of the catalyst may have occupied all its active sites, and therefore no more adsorption was observed.

Effect of Increasing g-C$_3$N$_4$/CeO$_2$ NCs Concentrations for OFX Removals in Pharmaceutical Industry Wastewater during Photocatalytic Degradation Process

Increasing g-C$_3$N$_4$/CeO$_2$ NCs concentrations (1 mg/l, 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l and 10 mg/l) were operated at 20 mg/l OFX, at 150 W UV-Vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0, at 25°C, respectively (Figure 9). 54.5%, 68.1%, 75.8%, 87.3% and 92.1% OFX removal efficiencies were obtained at 1 mg/l, 2 mg/l, 4 mg/l, 6 mg/l and 10 mg/l g-C$_3$N$_4$/CeO$_2$ NCs concentrations, respectively, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0, at 25°C, respectively (Figure 9). The maximum 99% OFX removal efficiency was measured to 8 mg/l g-C$_3$N$_4$/CeO$_2$ NCs with photocatalytic degradation process in pharmaceutical industry wastewater, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 9).

Effect of Different g-C$_3$N$_4$/CeO$_2$ NCs Mass Ratios for OFX Removals in Pharmaceutical Industry Wastewater during Photocatalytic Degradation Process

Different g-C$_3$N$_4$/CeO$_2$ mass ratios (5/5wt, 6/4wt, 7/3wt, 8/2wt, 9/1wt, 1/9wt, 2/8wt, 3/7wt and 4/6wt, respectively) were examined for OFX removal in pharmaceutical industry wastewater during photocatalytic degradation process, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 10). 97.5%, 96.2%, 94%, 93.8%, 89.2%, 86.2% and 80.1% OFX removal efficiencies were measured at 2/8wt g-C$_3$N$_4$/CeO$_2$ NCs mass ratio, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 10).

Effect of Different Recycle Times for OFX Removals in Pharmaceutical Industry Wastewater during Photocatalytic Degradation Process

Different recycle times (1., 2., 3., 4., 5., 6. and 7.) were operated for OFX removals in pharmaceutical industry wastewater during photocatalytic degradation process, at 20 mg/l OFX, 8 mg/l g-C$_3$N$_4$/CeO$_2$ NCs, at 2/8wt g-C$_3$N$_4$/CeO$_2$ NCs mass ratio, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 11). 97.5%, 96.2%, 94%, 93.8%, 89.2%, 86.2% and 80.1% OFX removal efficiencies were measured after 2. recycle time, 3. recycle...

Figure 8: Effect of increasing OFX concentrations for OFX removal in pharmaceutical industry wastewater during photocatalytic degradation process, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

Figure 9: Effect of increasing g-C$_3$N$_4$/CeO$_2$ NCs concentrations for OFX removal in pharmaceutical industry wastewater during photocatalytic degradation process, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

Figure 10: Effect of different g-C$_3$N$_4$/CeO$_2$ NCs mass ratios for OFX removal in pharmaceutical industry wastewater during photocatalytic degradation process, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.
time, 4. recycle time, 5. recycle time, 6. recycle time and 7. recycle time, respectively, at 20 mg/l OFX, 8 mg/l g-C3N4/CeO2 NCs, at 2/8 wt g-C3N4/CeO2 NCs mass ratio, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 11). The maximum 99% OFX removal efficiency was measured in pharmaceutical industry wastewater during photocatalytic degradation process, after 1. recycle time, at 20 mg/l OFX, 8 mg/l g-C3N4/CeO2 NCs, at 2/8 wt g-C3N4/CeO2 NCs mass ratio, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively (Figure 11).

Acute Toxicity Assays

Effect of Increasing OFX Concentrations on the Microtox (Aliivibrio fischeri or Vibrio fischeri) Acute Toxicity Removal Efficiencies in Pharmaceutical Industry Wastewater at Increasing Photocatalytic Degradation Time and Temperature

In Microtox with Aliivibrio fischeri (also called Vibrio fischeri) acute toxicity test, the initial EC90 values at pH=7.0 was found as 825 mg/l at 25°C (Table 3: SET 1). After 60 min, 120 min and 180 min photocatalytic degradation time, the EC90 values decreased to EC90=414 mg/l to EC90=236 mg/l and to EC90=165 mg/l in OFX=20 mg/l at 30°C (Table 3: SET 3). The Microtox (Aliivibrio fischeri) acute toxicity removal efficiencies were 40.86%, 79.75% and 90.86% after 60 min, 120 min and 180 min, respectively, in OFX=20 mg/l and at 30°C (Table 3: SET 3).

The EC90 values decreased to EC90, to EC90 and to EC90 after 60 min, 120 min and 180 min, respectively, in OFX=20 mg/l at 60°C (Table 3: SET 3). The EC90, the EC90 and the EC90 were measured as 550 mg/l, 540 mg/l and 500 mg/l, respectively, in OFX=20 mg/l at 60°C. The toxicity removal efficiencies were 46.41%, 85.30% and 96.41% after 60 min, 120 min and 180 min, respectively, in OFX=20 mg/l at 60°C (Table 3: SET 3).

The EC90 values decreased to EC90, to EC90 and to EC90 after 60 min, 120 min and 180 min, respectively, in OFX=20 mg/l and at 30°C (Table 3: SET 3). The Microtox (Aliivibrio fischeri) acute toxicity removal yield was found in OFX=20 mg/l after 180 min and at 60°C (Table 3: SET 3).

The EC90 values decreased to EC90=422 mg/l to EC90=241 mg/l and to EC90=168 mg/l after 60 min, 120 min and 180 min, respectively, in OFX=5 mg/l at 30°C (Table 3: SET 3). The EC90 values decreased to EC90=421 mg/l to EC90=239 mg/l and to EC90=167 mg/l after 60 min, 120 min and 180 min, respectively, in OFX=10 mg/l at 30°C. The EC90 values decreased to EC90=408 mg/l to EC90=230 mg/l and to EC90=162 mg/l after 60 min, 120 min and 180 min, respectively, in OFX=40 mg/l at 30°C. The Microtox (Aliivibrio fischeri or Vibrio fischeri) acute toxicity removals were 85.30%, 85.28% and 79.75% in 5 mg/l, 10 mg/l and 40 mg/l OFX, respectively, after 180 min, at 30°C. It was obtained an inhibition effect of OFX=40 mg/l to Vibrio fischeri after 180 min and at 30°C (Table 3: SET 3).

The EC90 values decreased to EC90=419 mg/l to EC90=266 mg/l and to EC90=150 mg/l after 60 min, 120 min and 180 min, respectively, in OFX=5 mg/l at 60°C (Table 3: SET 3). The EC90 values decreased to EC90=414 mg/l to EC90=232 mg/l and to EC90=161 mg/l after 60 min, 120 min and 180 min, respectively, in OFX=10 mg/l at 60°C. The EC90 values decreased to EC90=403 mg/l to EC90=218 mg/l and to EC90=148 mg/l after 180 min, at 60°C, respectively.

Table 3: Effect of increasing OFX concentrations on Microtox (Aliivibrio fischeri) acute toxicity in pharmaceutical industry wastewater after photocatalytic degradation process, at 30°C and at 60°C, respectively.

<table>
<thead>
<tr>
<th>No</th>
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<th>OFX (mg/l)</th>
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</table>

* EC values were calculated based on CODdis (mg/l).
values decreased to $EC_{27}=450$ mg/l to $EC_{32}=175$ mg/l and to $EC_{32}=120$ mg/l after 60 min, 120 min and 180 min, respectively, in OFX=40 mg/l at 60°C. The toxicity removal efficiencies were 72.22%, 72.56% and 63.21% in 5 mg/l, 10 mg/l and 40 mg/l OFX, respectively, after 180 min and at 30°C. It was observed an inhibition effect of OFX=40 mg/l to Daphnia magna after 180 min and at 30°C (Table 4: SET 3).

Toxicity removals were 83.06%, 92.65% and 73.11% in 5 mg/l, 10 mg/l and 40 mg/l OFX, respectively, after 180 min and at 60°C. It was observed an inhibition effect of OFX=40 mg/l to Daphnia magna after 180 min and at 60°C (Table 4: SET 3).

Increasing the OFX concentrations from 5 mg/l to 40 mg/l did not have a positive effect on the decrease of $EC_{50}$ values as shown in Table 4 at SET 3. OFX concentrations > 20 mg/l decreased the acute toxicity removals by hindering the photocatalytic degradation process. Similarly, a significant contribution of increasing OFX concentration to acute toxicity removal at 60°C after 180 min of photocatalytic degradation time was not observed. Low toxicity removals found at high OFX concentrations could be attributed to their detrimental effect on the Daphnia magna (Table 4: SET 3).

### Direct Effects of OFX Concentrations on the Acute Toxicity of Microtox (Alivibrio fischeri) and Daphnia magna without Pharmaceutical Industry Wastewater after Photocatalytic Degradation Process

The acute toxicity test was performed in the samples containing 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l OFX concentrations, at 25°C, 30°C and 60°C.

<table>
<thead>
<tr>
<th>No</th>
<th>Parameters</th>
<th>Daphnia magna Acute Toxicity Values, *EC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*EC</td>
</tr>
<tr>
<td>1</td>
<td>Raw ww, control</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>30°C</td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*EC</td>
</tr>
<tr>
<td>2</td>
<td>Raw ww, control</td>
<td>850</td>
</tr>
<tr>
<td>3</td>
<td>OFX=5 mg/l</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>OFX=10 mg/l</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>OFX=20 mg/l</td>
<td>850</td>
</tr>
<tr>
<td></td>
<td>OFX=40 mg/l</td>
<td>850</td>
</tr>
</tbody>
</table>

*EC values were calculated based on COD$_{dis}$ (mg/l).
room temperature. In order to detect the direct responses of Microtox (Aliivibrio fischeri or Vibrio fischeri) and Daphnia magna to the increasing OFX concentrations the toxicity test were performed without pharmaceutical industry wastewater after photocatalytic degradation process, at 25°C room temperature. The initial EC values and the EC values were measured in the samples containing increasing OFX concentrations after 180 min photocatalytic degradation time. Table 5 showed the responses of Microtox (Aliivibrio fischeri or Vibrio fischeri) and Daphnia magna to increasing OFX concentrations.

The acute toxicity originating only from 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l OFX were found to be low (Table 5). 5 mg/l OFX did not exhibited toxicity to Aliivibrio fischeri (or Vibrio fischeri) and Daphnia magna before and after 180 min photocatalytic degradation time. The toxicity attributed to the 10 mg/l, 20 mg/l and 40 mg/l OFX were found to be low in the samples without pharmaceutical industry wastewater after photocatalytic degradation process for the test organisms mentioned above. The acute toxicity originated from the OFX decreased significantly to EC2, EC4 and EC6 after 180 min photocatalytic degradation time. Therefore, it can be concluded that the toxicity originating from the OFX is not significant and the real acute toxicity throughout photocatalytic degradation process was attributed to the pharmaceutical industry wastewater, to their metabolites and to the photocatalytic degradation by-products (Table 5).

The Comparison with Other Scientific Studies in the Literature

Comparison of our study "The use of a novel graphitic carbon nitride/cerium dioxide (g-C3N4/CeO2) nanocomposites for the ofloxacin removal by photocatalytic degradation in pharmaceutical industry wastewaters and the evaluation of microtox (Aliivibrio fischeri) and Daphnia magna acute toxicity assays" with other scientific studies in the literature is summaried at Table 6 [175-182].

Conclusions

The maximum 99% OFX removal efficiency was obtained during photocatalytic degradation process in pharmaceutical industry wastewater, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

Table 5: The responses of Microtox (Aliivibrio fischeri or Vibrio fischeri) and Daphnia magna acute toxicity tests in addition of increasing OFX concentrations within pharmaceutical industry wastewater during photocatalytic degradation process after 180 min photocatalytic degradation time, at 25°C room temperature.

<table>
<thead>
<tr>
<th>OFX Conc. (mg/l)</th>
<th>Microtox (Aliivibrio fischeri or Vibrio fischeri) Acute Toxicity Test</th>
<th>Daphnia magna Acute Toxicity Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Acute Toxicity EC50 Value (mg/l)</td>
<td>Inhibitions after 180 min photocatalytic degradation time</td>
</tr>
<tr>
<td>5</td>
<td>EC2=24</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>EC7=99</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>EC14=19</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>EC21=19</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 6: The Comparison with other Scientific Studies in the Literature.
The maximum 99% OFX removal efficiency was found with photocatalytic degradation process in pharmaceutical industry wastewater, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

The maximum 99% OFX removal efficiency was measured to 8 mg/l g-C₃N₄/CeO₂ NCs with photocatalytic degradation process in pharmaceutical industry wastewater, at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

The maximum 99% OFX removal efficiency was measured at 2/8wt g-C₃N₄/CeO₂ NCs mass ratios at 20 mg/l OFX, at 300 W UV-vis light irradiation power, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

The maximum 99% OFX removal efficiency was measured in pharmaceutical industry wastewater during photocatalytic degradation process, after 1. recycle time, at 20 mg/l OFX, 8 mg/l g-C₃N₄/CeO₂ NCs, at 2/8wt g-C₃N₄/CeO₂ NCs mass ratio, after 180 min photocatalytic degradation time, at pH=6.0 and at 25°C, respectively.

96.41% maximum Microtox (Aliivibrio fischeri) acute toxicity removal yield was found in OFX=20 mg/l after 180 min and at 60°C. It was observed an inhibition effect of OFX=40 mg/l to Microtox with Vibrio fischeri after 180 min photocatalytic degradation time and at 60°C. OFX concentrations > 20 mg/l decreased the acute toxicity removals by hindering the photocatalytic degradation process. Similarly, a significant contribution of increasing OFX concentrations to acute toxicity removal at 60°C after 180 min photocatalytic degradation time was not observed. Finally, it can be concluded that the toxicity originating from the OFX is not significant and the real acute toxicity throughout photocatalytic degradation process was attributed to the pharmaceutical industry wastewater, to their metabolites and to the photocatalytic degradation process by-products.

As a result, the a novel g-C₃N₄/CeO₂ NCs photocatalyst during photocatalytic degradation process in pharmaceutical industry wastewater was stable in harsh environments such as acidic, alkaline, saline, and then was still effective process. When the amount of contaminant was increased, the a novel g-C₃N₄/CeO₂ NCs photocatalyst during photocatalytic degradation process performance was still considerable. The synthesis and optimization of g-C₃N₄/CeO₂ heterostructure photocatalyst provides insights into the effects of preparation conditions on the material's characteristics and performance, as well as the application of the effectively designed photocatalyst in the removal of antibiotics, which can potentially be deployed for purifying wastewater, especially pharmaceutical wastewater. Finally, the combination of a simple, easy operation preparation process, excellent performance and cost effective, makes this a novel g-C₃N₄/CeO₂ NCs a promising option during photocatalytic degradation process in pharmaceutical industry wastewater treatment.

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**References**


Barry S (2019) Dangerously high levels of antibiotics found in world’s major rivers, says study. World news global study 1: 1-10.


Mady AH, Baynosa ML, Tuma D, Shim JJ. (2019) Heterogeneous activation of peroxymonosulfate by a novel magnetic 3D gamma-MnO2@ZnFe2O4/GO nanohybrid as a robust catalyst for phenol degradation. Applied Catalysis B: Environmental 244: 946-956.


Wang ZT, Xu JL, Zhou H, Zhang X (2019a) Facile synthesis of Zn(II)-doped g-C3N4 and their enhanced photocatalytic activity under visible light irradiation. Rare Met 38: 459-467. [crossref]


Delia Teresa Sponza (2023) The Use of a Novel Graphitic Carbon Nitride/Cerium Dioxide (g-C3N4/CeO2) Nanocomposites for the Ofloxacin Removal by Photocatalytic Degradation in Pharmaceutical Industry Wastewaters and the Evaluation of Microtox (Aliislibro Fischeri) and Daphnia Magna Acute Toxicity Assays.


Citation: