



## Photocatalytic degradation and mineralization of microcystin-LR under UV-A, solar and visible light using nanostructured nitrogen doped TiO<sub>2</sub>

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### ABSTRACT

In an attempt to face serious environmental hazards, the degradation of microcystin-LR (MC-LR), one of the most common and more toxic water soluble cyanotoxin compounds released by cyanobacteria blooms, was investigated using nitrogen doped TiO<sub>2</sub> (N-TiO<sub>2</sub>) photocatalyst, under UV-A, solar and visible light. Commercial Degussa P25 TiO<sub>2</sub>, Kronos and reference TiO<sub>2</sub> nanopowders were used for comparison. It was found that under UV-A irradiation, all photocatalysts were effective in toxin elimination. The higher MC-LR degradation (99%) was observed with Degussa P25 TiO<sub>2</sub> followed by N-TiO<sub>2</sub> with 96% toxin destruction after 20 min of illumination. Under solar light illumination, N-TiO<sub>2</sub> nanocatalyst exhibits similar photocatalytic activity with that of commercially available materials such as Degussa P25 and Kronos TiO<sub>2</sub> for the destruction of MC-LR. Upon irradiation with visible light Degussa P25 practically did not show any response, while the N-TiO<sub>2</sub> displayed remarkable photocatalytic efficiency. In addition, it has been shown that photodegradation products did not present any significant protein phosphatase inhibition activity, proving that toxicity is proportional only to the remaining MC-LR in solution. Finally, total organic carbon (TOC) and inorganic ions (NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) determinations confirmed that complete photocatalytic mineralization of MC-LR was achieved under both UV-A and solar light.

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### 1. Introduction

Cyanobacteria (blue-green algae) are considered an important water quality problem, since several genera can produce toxins, called cyanotoxins that are harmful to human health. Blooms of toxic cyanobacteria have been reported worldwide and several incidents of wild and domestic animal poisoning as well as human injury and death have been reported [1].

Hepatotoxic cyclic peptide toxins (microcystins, MCs) are the most widespread cyanotoxins that are present in diverse environments. MCs are cyclic heptapeptides that are produced by cyanobacteria belonging to the genera *Microcystis*, *Anabaena*, *Plankothrix*, *Nostoc* and *Snowella*, and it is likely that more genera of cyanobacteria will be identified as microcystin producers in the future [2]. About 80 MC variants have been identified so far in natural water samples and cyanobacterial cultures, with microcystin-LR (MC-LR) being the most common and most toxic variant [3].

During the last years there is a growing concern regarding the health effects of MCs, because they act as tumor promoters, through the inhibition of protein phosphatases 1 and 2A, which play a key role in cell regulation [4]. In response to this concern, WHO has recommended for provisional adoption the value of 1 µg L<sup>-1</sup> as a Guideline Value for MC-LR concentration in drinking water [2].

A variety of traditional water treatment methods, including coagulation/sedimentation [5], activated carbon adsorption [6] and membrane separation [7], have been attempted for the removal of cyanotoxins, but with limited success.

Consequently, research on new methods for water purification develops in different ways, including chemical, electrochemical or photochemical processes. Among all kinds of water purification techniques, oxidative degradation methods, using electromagnetic radiation have been receiving increased attention for the detoxification of the aquatic environment. They are generally referred to as advanced oxidation processes (AOP) and mainly involve UV light in the presence of hydrogen peroxide or ozone, and UV and near visible light in the presence of TiO<sub>2</sub> [8–12]. These methods lead to mineralization of the organic pollutants and are mainly based on the generation of electron/hole pairs and highly oxidizing OH radicals.

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Previous studies have demonstrated that TiO<sub>2</sub> photocatalysis [13–20] can effectively destroy cyanotoxins in aqueous solution and that hydroxyl radical is responsible for a significant fraction of the observed degradation. Emphasis was given to the mechanistic understanding of the process by identification of the intermediates formed during photocatalysis [21]. To the best of our knowledge there are only few studies concerning the MC-LR photocatalytic mineralization [17,22,23].

The assessment of pollutant disappearance is not sufficient to ensure the absence of residual products since the photocatalytic treatment is a complex procedure leading to the formation of degradation products, which in some cases may be more toxic and stable than the parent compound. Therefore careful analytical and/or toxicological monitoring using various techniques is important in order to control all transformation steps, but also the final degradation products.

Photocatalysis in the presence of TiO<sub>2</sub> although appears to degrade effectively MC-LR, it requires UV radiation ( $\lambda < 400$  nm) to overcome its wide band gap energy ( $\sim 3.2$  eV for anatase phase) for photocatalytic activation. To overcome this limitation, doping of TiO<sub>2</sub>, to reduce its band gap energy requirements, has been performed. Among other procedures non-metal doping with nitrogen (N) gives new materials with good photocatalytic performance in the visible light [24–26].

Highly efficient N-TiO<sub>2</sub> nanoparticles with enhanced structural properties and high photocatalytic activity under visible light irradiation have been recently prepared [27]. However, its response in MC-LR destruction and mineralization has not been investigated.

According to recent studies, nitrogen doping of TiO<sub>2</sub>, at substitutional on the oxygen lattice sites, forms nitride (Ti–N), or oxynitride (O–Ti–N) arrangements that introduce energy states above the valence band of titania due to N 2p or mixing of the N 2p with O 2p states. Also, oxoanion N–O species at interstitial lattice sites can be formed creating localized intergap states. Thus, in both nitrogen doping configurations, energy states are formed inside the titania band gap accounting for the red shift of the energy gap and the induced visible light photocatalytic activity [27].

In this study we report for the first time the photocatalytic degradation of MC-LR using UV-A, solar and visible light irradiation in the presence of a new nanostructured N-TiO<sub>2</sub> photocatalyst. Commercial Degussa P25 TiO<sub>2</sub>, Kronos and reference TiO<sub>2</sub> nanopowders were used for comparison. In addition, the mineralization extent has been evaluated by using TOC and inorganic ions (NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) determinations. Residual toxicity was assessed by the protein phosphatase inhibition assay (PPIA).

## 2. Experimental

### 2.1. Materials

N-doped TiO<sub>2</sub> powders (N-TiO<sub>2</sub>) were synthesized as the hydrolysis condensation product of tetrabutyl titanate reaction with urea [27]. The reference TiO<sub>2</sub> nanopowder was prepared without adding urea. Commercial Degussa P25 TiO<sub>2</sub> (Degussa AG, Germany), Kronos vlp-7000 (Kronos Titan GmbH, Germany) and reference TiO<sub>2</sub> nanopowders were used as standard materials for comparison purposes. MC-LR was purchased from Abraxis, USA.

### 2.2. Instrumentation

Irradiation in UV-A (320–400 nm) was performed with a laboratory constructed “illumination box” equipped with four F15W/T8 black light tubes (Sylvania GTE, USA). The maximum emission of these tubes is around 365 nm, emitting 71.7 mW cm<sup>-2</sup> at a distance of 25 cm.

A set of nine high power Luxeon dimmable light emitting diodes (LXML-PR01-0425 Luxeon® Rebel high power LED-max power of each LED equal to 425 mW) emitting at the narrow royal blue spectral region, in between 440 and 460 nm, where the N-doped TiO<sub>2</sub> presents significant absorption, was used as source for visible light irradiation. The LEDs modulus produces a homogenous beam with a power density of 3 mW cm<sup>-2</sup> at a distance of 7 cm from the photocatalytic cell.

Visible light irradiation covering the whole visible range (400–700 nm) was provided by an Oriel photolysis apparatus (Photomax) equipped with an Oriel 150 W Xe arc lamp, a cool water circulating liquid filter to absorb the near IR radiation and a 435 nm cut-off filter exhibiting a zero transmittance below 410 nm. The light intensity was measured to be 155 mW cm<sup>-2</sup> by a power meter (Ealing Electro-Optics). When a 420 nm cut-off filter was used instead of the 435 nm cut-off one, a transmittance of 3.25% was observed below 400 nm giving rise to not reliable results, due to the exposure of photocatalysts under UV-A irradiation.

Irradiation experiments with solar light were carried out with the same apparatus (Photomax) equipped with an Oriel AM1.5 G air mass filter. This filter corrects the output of the 150 W xenon arc lamp to approximate the solar spectrum when the sun is at a zenith angle of 48.2°. The light intensity was measured to be 115 mW cm<sup>-2</sup> by a power meter (Ealing Electro-Optics).

In all cases a borosilicate glass vessel reactor was sealed and placed inside a water thermostated jacket in order to prevent evaporation and retain the sample temperature constant at 25 °C during the whole period of irradiation.

### 2.3. Analytical procedures

MC-LR degradation was monitored by HPLC, using an HPLC apparatus consisted of a Waters (Milford, MA, USA) Model 600E pump associated with a Waters Model 600 gradient controller, a Rheodyne (Cotati, CA, USA) Model 7725i sample injector equipped with 20  $\mu$ L sample loop, an Agilent Eclipse XDC-C18 analytical column (15 cm  $\times$  4.6 mm ID, 3.5  $\mu$ m) and a Waters Model 486 tunable absorbance detector, set at 238 nm, controlled by the Millennium (Waters) software. Elution solvents used was acetonitrile (A)–water (B) both containing 0.05% TFA, with gradient elution mode, an injection volume of 20  $\mu$ L, sample manually injected and with ambient column temperature. The gradient elution program was as follows: 35% A–65% B at 0.00 min, 70% A at 2.00 min, 100% A at 2.10 min, 35% A at 3.10 min, at a flow rate of 1.2 mL min<sup>-1</sup>. Samples were filtered through Millex PVDF Durapore–GF 13 mm 0.22  $\mu$ m (low protein binding) filters.

For the monitoring of inorganic ions (NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) an Ionic Chromatograph of Metrom which consists of an electrochemical detector 761 Compact IC and Metrosep C2 150 and Anion Dual 2 analytical columns for cations and anions, respectively, was used.

Total organic carbon (TOC) was measured on filtered samples using a Shimadzu TOC-5000A. Calibration of the TOC analyser was achieved using potassium hydrogen phthalate standards. Toxicity was assessed by the protein phosphatase inhibition assay (PPIA) using the MicroCystest kit (Zeu-Immunotec S.L., Spain) in 96-well microtiter plate format.

N/No. of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> is the ratio between the concentration (*M*) of each ion as determined at various time intervals during MC-LR photocatalytic degradation divided by the concentration (*M*) of nitrogen based on MC-LR chemical structure and initial concentration.

In the case of total nitrogen, *N* represents the summary of all ions concentrations (*M*) at various time intervals of illumination.

#### 2.4. Photocatalytic experiments

In a typical experiment, 5 mL of aqueous MC-LR solution ( $10 \text{ mg L}^{-1}$ ) containing the photocatalyst (N-TiO<sub>2</sub>, commercial Degussa P25 TiO<sub>2</sub>, Kronos vlp-7000 and reference TiO<sub>2</sub> nanopowder at  $200 \text{ mg L}^{-1}$ ) were added to a cylindrical pyrex cell, oxygenated for 20 min and covered air tightly with a serum cap. Photolysed solutions were freshly prepared in MilliQ-grade water for every experiment and used after 4 h equilibration in the dark under stirring. The initial pH was 5.7. Photolysis was performed at ambient temperature in the photolysis apparatus. The solutions were magnetically stirred throughout the experiment. Analysis of the photolysed solutions was performed after filtration. All experiments are performed in triplicate and the error bars on figures represent the mean  $\pm$  SD of the three separate measurements. The initial rates of MC-LR degradation were calculated from the slope of the curve obtained for the first 30% of the MC-LR destruction.

### 3. Results and discussion

#### 3.1. Photocatalytic degradation/mineralization of MC-LR under UV-A light

Illumination of an aqueous solution of MC-LR with  $\lambda_{\text{max}} = 365 \text{ nm}$  in the presence of commercial Degussa P25 TiO<sub>2</sub>, Kronos, N-TiO<sub>2</sub>, and reference TiO<sub>2</sub> nanopowders result in photodegradation of substrate, whereas experiments in the absence of photocatalyst show that direct photolysis is negligible (Fig. 1). These results are in agreement with those reported by others concerning commercial Degussa P25 TiO<sub>2</sub> [15]. As can be seen in Fig. 1, under UV-A irradiation all photocatalysts were effective in toxin elimination. The higher MC-LR degradation (99%) was observed with Degussa P25 TiO<sub>2</sub> followed by N-TiO<sub>2</sub>, reference-TiO<sub>2</sub>, and Kronos TiO<sub>2</sub> with 96, 95 and 89% toxin destruction, respectively, after 20 min of irradiation. Under the experimental conditions used, the initial degradation rates were  $3.00 \pm 0.23$ ,  $0.60 \pm 0.03$ ,  $1.97 \pm 0.64$  and  $1.90 \pm 0.59 \text{ } \mu\text{M min}^{-1}$  for Degussa P25 TiO<sub>2</sub>, Kronos TiO<sub>2</sub>, reference-TiO<sub>2</sub>, and N-TiO<sub>2</sub> respectively.

Fig. 2 illustrates the influence of catalyst concentration on the MC-LR degradation in oxygenated solutions. Experiments have been performed in presence of Degussa P25 TiO<sub>2</sub> at three different concentrations, i.e. 50, 100 and 200 ppm.

It can be seen that increase of the catalyst initial concentration from 50 to 100 ppm results in roughly two-fold increase of the MC-LR degradation rate and disappearance of MC-LR in a shorter

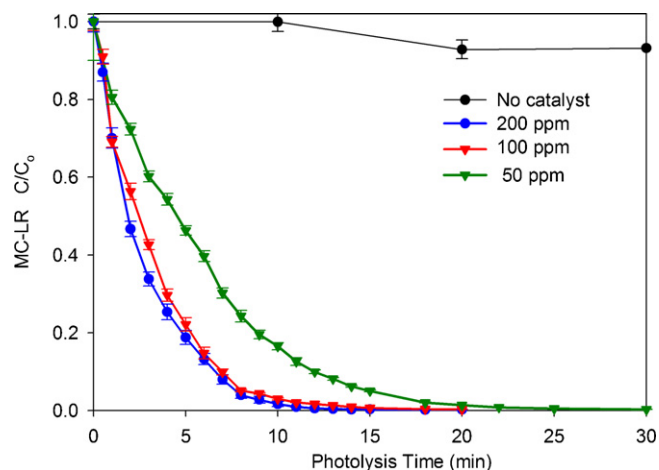


Fig. 2. Degradation of MC-LR under UV-A light using different concentrations of Degussa P25 TiO<sub>2</sub>.

irradiation time (20 instead of 30 min). Further increase of the catalyst initial concentration to 200 ppm has also a slight influence in MC-LR degradation, so this concentration has been used as initial concentration for all tested materials in order to compare their photocatalytic effectiveness towards toxin destruction.

The mechanism of the light induced reactions occurring on the surface of the TiO<sub>2</sub> has been extensively discussed [28]. The degradation of MC-LR seems to occur through a similar process previously described for a great number of organic pollutants that involves attack of the substrate by radical species, the OH radical being the most powerful oxidant [21,29,30].

Liu et al. [29] were the first to study the degradation pathway of MC-LR with TiO<sub>2</sub> photocatalysis in the presence of hydrogen peroxide at acidic pH (pH 4). According to their findings, OH radical attack on the conjugated double bonds of the Adda amino acid produces dihydroxylated products that upon further oxidation yield the corresponding carboxylic acids. Photocatalysis also hydrolyzes the peptide bond on the ring structure of microcystin to form linear structures although this appeared to be a minor pathway.

Antoniou et al. [21,30] indicated that MC-LR degradation is initiated at four sites of the toxin; three on the Adda amino acid (aromatic ring, methoxy group, and conjugated double bonds) and one on the cyclic structure (Mdha amino acid), with the conjugated diene bonds being the most affected site. Most of the identified intermediates had an intact cyclic structure; however, towards the end of the treatment, linear intermediates were isolated as well. The identification of intermediates in these studies has been performed using liquid chromatography/mass spectrometry (LC/MS and MS/MS) [21,29,30].

To the best of our knowledge, the degradation process has been studied only during limited time of irradiation. Under these conditions, less than 20% mineralization has been achieved, although after 60 min of photocatalysis the degradation products represent less than 1% of the starting material [29]. The explanation given to this apparent discrepancy is that products with mass below 400 Da were difficult to be detected by LC/MS because of overwhelming noise signals. However, the MS/MS data, the breakdown curves and the proposed mechanisms of formation of those identified intermediates are very helpful in giving an idea for the structure of unidentified degradation products [21].

In this study, prolonged illumination of an aqueous solution of MC-LR in presence of Degussa P25 TiO<sub>2</sub> has been performed. An almost total mineralization of the substrate occurs, giving as final products CO<sub>2</sub> and inorganic ions (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) (Fig. 3). NO<sub>2</sub><sup>-</sup>, that is produced in traces during the process, finally is oxidized to NO<sub>3</sub><sup>-</sup>.

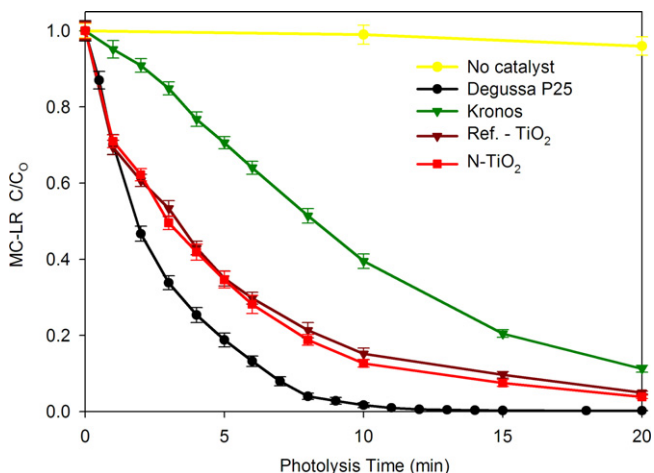
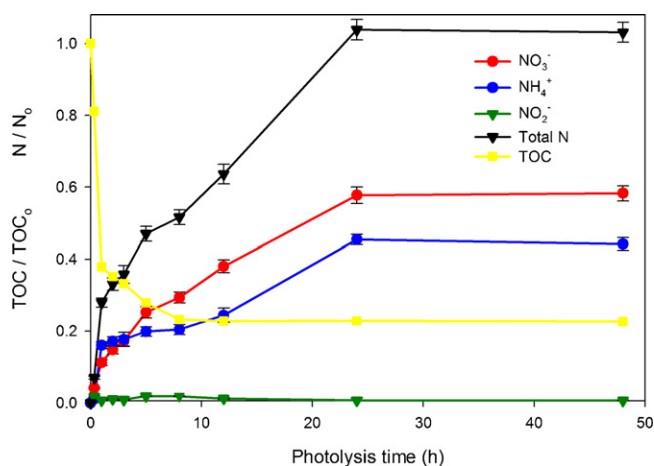


Fig. 1. Photocatalytic degradation of MC-LR under UV-A light in the presence of different TiO<sub>2</sub> based nanostructured materials.



**Fig. 3.** Carbon mineralization and time evolution of nitrate, nitrite, ammonium ions and total nitrogen during MC-LR degradation under UV-A irradiation in the presence of P25 TiO<sub>2</sub>.

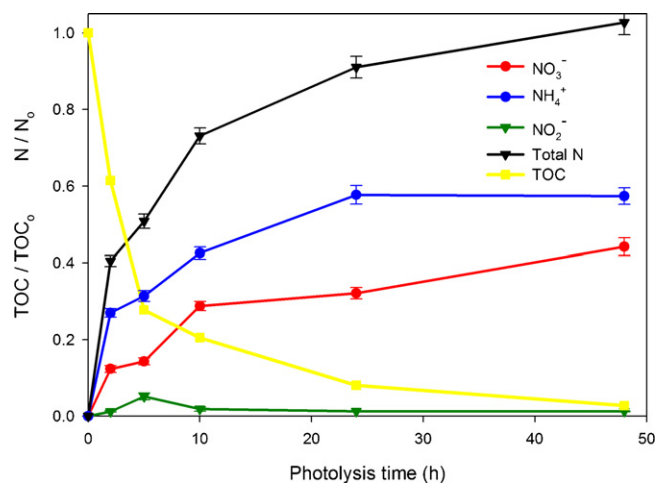
As can be seen in Fig. 3, after 8 h of irradiation ~80% of organic carbon and ~50% of organic nitrogen have been recovered, while degradation of MC-LR has been completed in less than 20 min (Fig. 1). The complete disappearance of the MC-LR in a much shorter time than the evolution of CO<sub>2</sub> and inorganic ions is due to the presence of organic intermediates before the complete mineralization of the substrate. The lack of ~20% on initial TOC value should be assigned to the still remaining nitrogen containing compounds, such as linear sequences of amino acids, similar to those identified, after intense photocatalysis, with TiO<sub>2</sub> [21,31]. Within these peptide derivatives and related structures, there are molecules containing C–N bonds, with or without H extractable atoms.

According to an overview on the fate of organic nitrogen in photocatalysis with TiO<sub>2</sub>, for structures with extractable H on carbon [32], the proposed mechanism of transformation occurs via an OH attack on the α-CH bond resulting in hydrogen extraction. Through further oxidation steps the nitrogen is finally released as ammonium ions. This general model is closely related to that for amino acids, peptides and proteins which has been deeply discussed [33,34].

Nitrate formation cannot be explained on the basis of the mechanism described above. When no extractable H exists on carbon, OH radical attack seems to occur on the nitrogen instead of the carbon atom. This is the case of guanidine and its derivatives, structural units of arginine in MC-LR, where OH attack occur on the amino group with the release of nitrogen mainly as nitrite, which is, very fast, oxidized into nitrate ions [35].

After 24 h of irradiation, the total nitrogen produced accounts for almost the stoichiometric quantity of the initial organic nitrogen. The remaining organic carbon ~20% can be attributed to small aliphatic hydrocarbon compounds (Fig. 3).

The ratio [NH<sub>4</sub><sup>+</sup>]/[NO<sub>3</sub><sup>-</sup>] which is characteristic of the substrate molecular structure was after 5 h of irradiation, 3:4. These results are in agreement with those reported for guanidine and methylguanidine derivatives, where the organic nitrogen is mainly released as nitrate ions [32,35]. The increased yield of nitrate ions can also be attributed to a secondary pathway proposed for amino acids photocatalytic degradation, which could occur through the release of an amino group as hydroxylamine from the parent molecule being then transformed into nitrate ions and acetic acid [34,36]. The acid can subsequently undergo oxidation via a photo-Kolbe type process (oxidation via electron transfer and decarboxylation) to generate carbon dioxide and formic acid, which also generates carbon dioxide via a similar process [34].



**Fig. 4.** Mineralization of MC-LR using N-TiO<sub>2</sub> under UV-A light.

In Fig. 4 the photocatalytic mineralization of MC-LR under UV-A irradiation in the presence of N-TiO<sub>2</sub> is presented. Based on TOC measurements, more than 70% has been degraded in 6 h, whereas almost complete mineralization has been achieved after illumination for 48 h.

At the same time period, the total nitrogen produced accounts for the stoichiometric quantity of the initial organic nitrogen. As comes out from the time evolution curves of nitrate and ammonium ions, the characteristic ratio [NH<sub>4</sub><sup>+</sup>]/[NO<sub>3</sub><sup>-</sup>] was changed to 3:2 after 10 h of irradiation.

The greater overall yield of NH<sub>4</sub><sup>+</sup> ions than the corresponding amount of NO<sub>3</sub><sup>-</sup> was attributed to the release of inorganic nitrogen from N-TiO<sub>2</sub> photocatalyst during the process. From blank experiments which are performed upon stirring and illumination of N-TiO<sub>2</sub> in the absence of MC-LR it was shown that after 2 h, nitrogen mainly as NH<sub>4</sub><sup>+</sup> was released corresponding to 25% of the initial organic nitrogen present in MC-LR.

Various model compounds such as amino acids, sorbic acid, 2-acetamidoacrylic acid have been used to mimic degradation of individual functional groups of MC-LR [34,35,37]. These results should be assessed carefully because MC-LR is not simply an assemblage of these small molecules. Finally, although there are many unclear points in understanding mineralization pathways, the complete mineralization of MC-LR by TiO<sub>2</sub> photocatalysts is clearly observed.

Assessment of the residual toxicity of MC-LR during the course of degradation was performed using PPIA. Experiments performed under UV illumination showed that for Degussa P25 and N-TiO<sub>2</sub> photocatalysts, toxicity is proportional only to the remaining MC-LR in solution, therefore degradation products present no significant protein phosphatase inhibition activity.

### 3.2. Photocatalytic degradation of MC-LR under solar light

Although photocatalytic degradation of MC-LR with undoped TiO<sub>2</sub> shows remarkable efficiency, it requires high cost UV radiation. Activation of TiO<sub>2</sub> under visible light can facilitate the development of promising processes for the remediation of contaminated water resources using solar light without complicated facilities for generating and introducing UV light. The reported spectrum of the employed N-TiO<sub>2</sub> photocatalyst [27] shows clearly extension of absorbance to the visible range (400–500 nm). Based on this evidence we have used this TiO<sub>2</sub> photocatalyst for toxin degradation under solar light irradiation.

The employed illumination apparatus provides a very close spectral match to solar spectra offering significant advantages such

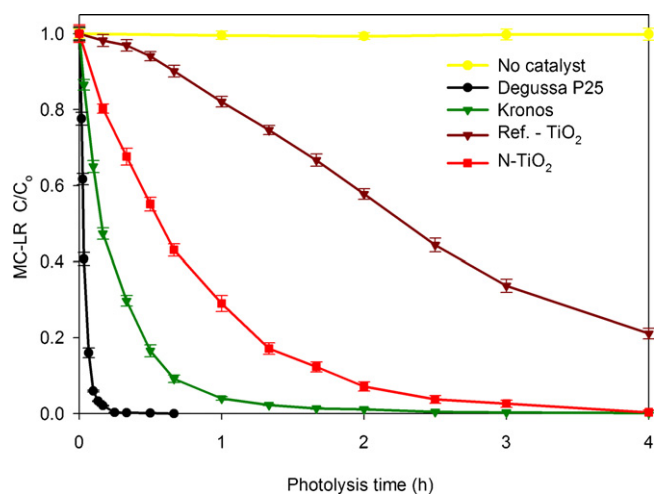


Fig. 5. Photocatalytic degradation of MC-LR under solar light irradiation (AM 1.5 G), in the presence of TiO<sub>2</sub> based materials.

as control of the local environmental parameters, repeated and comparable experimental conditions, unlimited availability of solar radiation etc. Under these experimental conditions, the nanostructured TiO<sub>2</sub> materials exhibited high photocatalytic activity as it is depicted in Fig. 5.

As can be seen N-TiO<sub>2</sub> nanocatalyst exhibits similar photocatalytic activity with that of commercially available materials such as Degussa P25 and Kronos TiO<sub>2</sub> for the destruction of MC-LR. Under solar light illumination, N-TiO<sub>2</sub> has shown complete elimination of MC-LR after irradiation for 4 h. The better performance of Degussa P25 TiO<sub>2</sub> seems to be due to the UV part of the solar light. Under the experimental conditions used, the initial degradation rates were  $2.91 \pm 0.32$ ,  $0.58 \pm 0.02$ ,  $0.033 \pm 0.002$  and  $0.16 \pm 0.02 \mu\text{M min}^{-1}$  for Degussa P25 TiO<sub>2</sub>, Kronos TiO<sub>2</sub>, reference-TiO<sub>2</sub>, and N-TiO<sub>2</sub>, respectively.

The mineralization of MC-LR using N-TiO<sub>2</sub> under solar light is presented in Fig. 6. Based on TOC measurements, after illumination for 48 h a mineralization extent of 85% has been achieved. The total nitrogen produced after 48 h of illumination accounts for the 80% of the initial organic nitrogen.

These results shown that the tested N-TiO<sub>2</sub> nanocatalyst exhibits high photocatalytic activity for the destruction of MC-LR under UV-A and solar irradiation. Under the employed experimental conditions the tested photocatalyst achieved complete degradation of MC-LR toxin. The similar behaviour of N-TiO<sub>2</sub> with

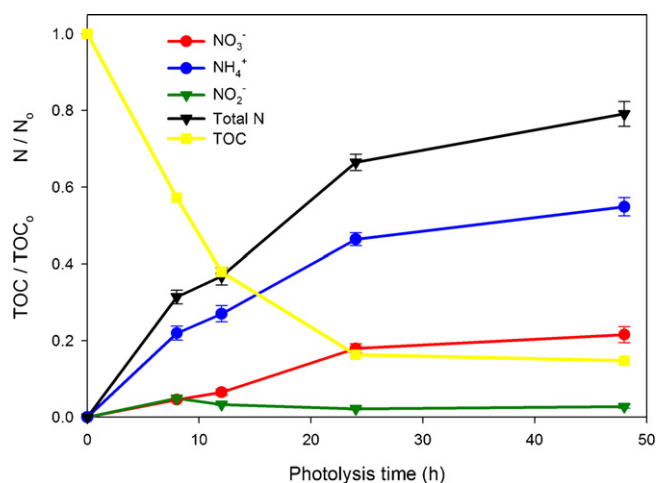


Fig. 6. Mineralization of MC-LR using N-TiO<sub>2</sub> under solar light.

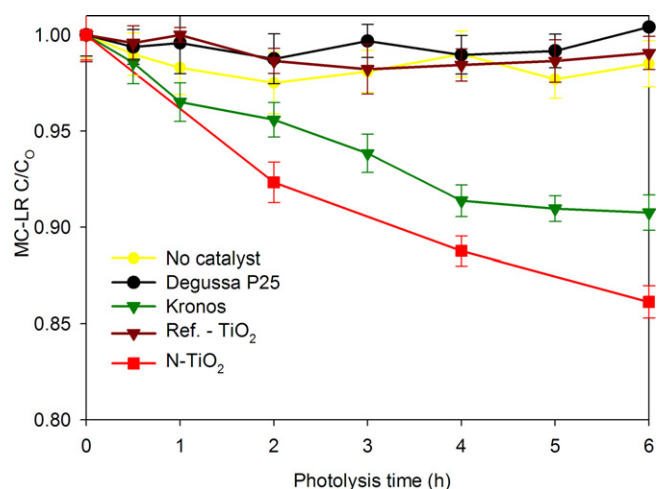


Fig. 7. Photocatalytic degradation of MC-LR under visible light (440–460 nm), in the presence of TiO<sub>2</sub> nanostructured materials.

that of Degussa P25 standard material suggests common degradation pathways mainly through the OH radical generation.

### 3.3. Photocatalytic degradation of MC-LR under visible light

In order to evaluate the photocatalytic efficiency of the new TiO<sub>2</sub> nanostructured materials a series of experiments have been performed under visible light irradiation employing different illumination apparatus.

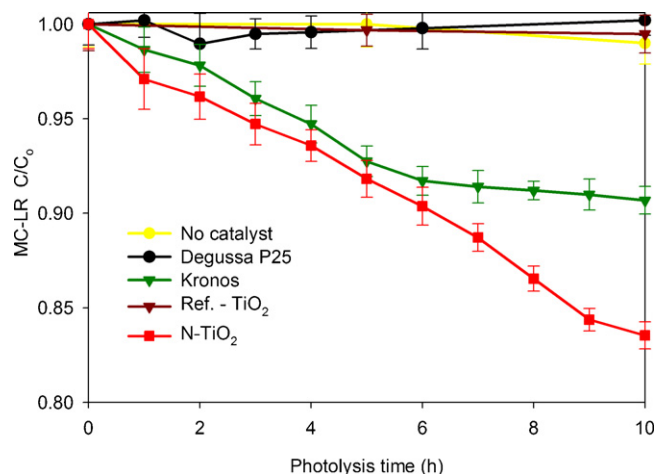
In the first case, a LEDs module emitting at 440–460 nm has been used. Under these experimental conditions, illumination of an aqueous solution of MC-LR in the presence of Degussa P25 and reference TiO<sub>2</sub> practically did not show any photocatalytic activity (Fig. 7) as these materials do not exhibit absorption of radiation with wavelengths greater than 410 nm. In contrast, illumination of MC-LR in the presence of Kronos TiO<sub>2</sub> and doped N-TiO<sub>2</sub> materials displayed a photocatalytic activity of 9 and 14% toxin degradation, respectively, after irradiation for 6 h (Fig. 7). These results show that the tested TiO<sub>2</sub> photocatalysts have been activated in the visible region of light and although the incident beam is very narrow, they exhibit improved photocatalytic activity with remarkable degradation of MC-LR.

Further evaluation of the new nanostructured TiO<sub>2</sub> photocatalysts for their activity towards MC-LR destruction has been performed by employing visible light radiation with wavelengths greater than 410 nm (Fig. 8).

As we can see in Fig. 8, the visible light activated TiO<sub>2</sub> photocatalysts (Kronos TiO<sub>2</sub>, N-TiO<sub>2</sub>) show remarkable activity in MC-LR degradation, while Degussa P25 and reference TiO<sub>2</sub> are completely inactive. Among the tested materials, N-TiO<sub>2</sub> shows the better photocatalytic activity for MC-LR degradation followed by Kronos TiO<sub>2</sub>. The commercial Kronos TiO<sub>2</sub> vlp-7000 is a C-doped visible light activated photocatalyst. C-doping involves substitution of oxygen by carbon atoms producing new energy states deep in the TiO<sub>2</sub> band gap, which are responsible for the visible light absorption [38,39].

Inactivity of Degussa P25 has been confirmed, as it absorbs light with  $\lambda < 410 \text{ nm}$  due to rutile phase, which accounts for 30% of Degussa P25 TiO<sub>2</sub> ( $E_{\text{bg}} = 3.0 \text{ eV}$ ) [40]. For that reason, the used cut-off filter for visible light should exhibit zero transmittance at wavelengths lower than 410 nm.

This behaviour of N-TiO<sub>2</sub> is attributed to a red-shift of the energy band gap to the visible range at about 2.3 eV that justifies visible light photocatalytic activity towards degradation of MC-LR [27]. Under the experimental conditions used, the initial degradation



**Fig. 8.** Photocatalytic degradation of MC-LR under visible light (435 nm cut-off filter), in the presence of TiO<sub>2</sub> based materials.

rates were  $2.70 \pm 0.10 \times 10^{-3}$  and  $1.67 \pm 0.20 \times 10^{-3} \mu\text{M min}^{-1}$  for N-TiO<sub>2</sub> and Kronos TiO<sub>2</sub>, respectively.

In order to test the stability of the N-TiO<sub>2</sub>, photocatalytic degradation experiments similar to the above were performed under visible light after washing and illuminating the TiO<sub>2</sub> photocatalyst. Results obtained were very similar to those presented in Fig. 8 indicating that N-TiO<sub>2</sub> photocatalyst can be reused.

#### 4. Conclusions

Visible light activated N-TiO<sub>2</sub> exhibits high photocatalytic activity for the destruction of MC-LR under UV-A and solar irradiation. Photodegradation proceeds to complete toxin mineralization giving CO<sub>2</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> as final products. Commercial Degussa P25 TiO<sub>2</sub>, Kronos and reference TiO<sub>2</sub> nanopowders were used for comparison.

Assessment of the residual toxicity of MC-LR during the course of degradation using PPIA under UV illumination showed that for Degussa P25 and N-TiO<sub>2</sub> photocatalysts, toxicity is proportional only to the remaining MC-LR in solution, therefore degradation products present no significant protein phosphatase inhibition activity.

N-TiO<sub>2</sub> nanocatalyst displayed a remarkable photocatalytic efficiency for toxin destruction upon irradiation under visible light, while Degussa P25 TiO<sub>2</sub> was totally inactive. These results indicate that the tested N-doped TiO<sub>2</sub> photocatalyst exhibits a remarkable photo-efficiency in the visible light that could be promising for the destruction of toxic organic pollutants in water, overcoming limitations concerning UV light utilization.

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