

## PHOTOCATALYTIC DEGRADATION OF CYANOTOXINS UNDER VISIBLE LIGHT; ELUCIDATION OF THE REACTION MECHANISM

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### ABSTRACT

Water pollution has become an environmental issue of ever increasing importance. In addition to conventional technologies used for purification of water, another type of treatment processes, commonly referred as advanced oxidation processes (AOPs) is emerging. Among AOPs, TiO<sub>2</sub> photocatalysis is considered to be very promising. The process efficiency is based on the ability of TiO<sub>2</sub> to generate highly reactive oxygen species (ROS) upon illumination with UV light, with hydroxyl radicals (HO<sup>•</sup>) being the main oxidative species. The main drawback of TiO<sub>2</sub> photocatalysis is the high energy need for photocatalytic activation. To overcome this limitation, doping of TiO<sub>2</sub> reduces its energy requirements, giving new materials with good photocatalytic performance in the visible light. Up to date, limited studies have been performed for the elucidation of the mechanism and degradation pathways followed in the process, being yet unclear.

Cyanobacteria are considered an important water quality problem, since several genera can produce toxins, called cyanotoxins that are harmful to human health. Among cyanotoxins, microcystin-LR (MC-LR) is the most common and toxic variant of the group of microcystins (MCs), while cylindrospermopsin (CYN) is an alkaloid neurotoxin that has been reported in water reservoirs and lakes worldwide. Geosmin (GSM) and 2-methylisoborneol (MIB), even though they are not considered as toxic, they give unpleasant taste and odor in water. Although the degradation of these compounds has already been studied under TiO<sub>2</sub>/UV light, the studies concerning their degradation under visible light photocatalysis are limited.

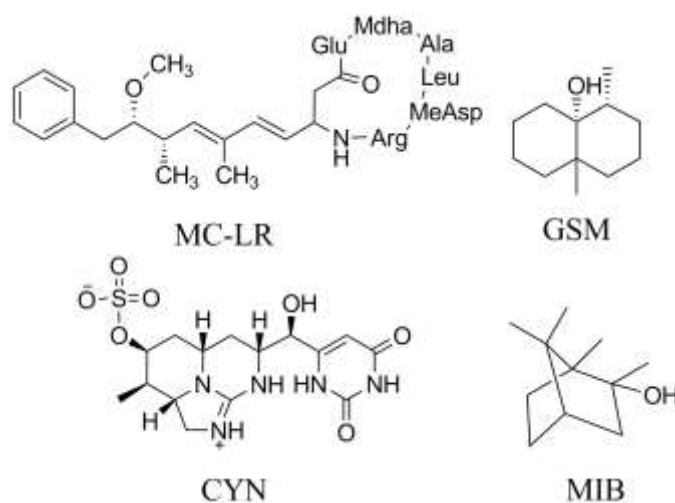
The main objective of this study was the photocatalytic degradation of MC-LR, CYN and ANA using C-doped TiO<sub>2</sub> (Kronos vlp-7000) under visible light and the elucidation of the mechanism followed. For that purpose, the identification of the intermediate products formed was achieved using LC-MS/MS analysis. In addition, identification of the main ROS formed during the process, was performed with EPR analysis. The approach employed was through the spin trapping agents 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), 5-tert-Butoxycarbonyl - 5- methyl -1- pyrroline- N-oxide (BMPO) and 5 - (Diethoxyphosphoryl) -5- methyl -1-pyrroline-N-oxide (DEPMPO), upon determination of their superoxide and the hydroxyl adducts.

**Keywords:** Visible Light Photocatalysis, Titanium Dioxide, EPR Spin Trapping, Reactive Oxygen Species, Cyanotoxins

### 1. Introduction

Cyanobacteria (blue-green algae) are considered an important water quality problem, since several genera can produce toxins, of different chemical structure called cyanotoxins that are harmful to human health. The heptapeptide structured microcystins (MCs) are the most widespread cyanotoxins that are present in diverse aqueous environments. MCs in drinking water can cause acute and chronic toxicity to humans with microcystin-LR (MC-LR) being the most common and most toxic variant of microcystins (Dawson 1998). Among the most important and potent cyanotoxins is the alkaloid like cylindrospermopsin (CYN) due to its serious health hazards and frequency of occurrence. The most commonly occurring off-odor compounds produced by

eutrophication and algal blooms are geosmin (GSM) and 2-methylisoborneol (MIB). They have strong earthy-muddy (GSM) and musty (MIB) odors and even though they are considered nontoxic their removal from water is necessary (I. H. Suffett *et al.* 1996).



**Figure 1:** Structures of MC-LR, CYN, GSM and MIB

Water purification has become an environmental issue of ever increasing importance. In addition to conventional technologies used for purification of water, another type of treatment process, commonly referred as advanced oxidation processes (AOPs) is emerging. AOPs destroy hazardous organic compounds instead of transferring them to another phase. Among AOPs, TiO<sub>2</sub> photocatalysis is considered to be highly promising. The process efficiency is based on the ability of TiO<sub>2</sub> to generate highly reactive oxygen species (ROS) upon illumination with UV light ( $\lambda \geq 400\text{nm}$ ). To overcome this limitation, doping of TiO<sub>2</sub>, to reduce its band gap energy requirements, is under investigation. Among other procedures non-metal doping with carbon (C) gives new materials with good photocatalytic performance in the visible light. C-doping e.g. Kronos vlp-7000, 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 (Goldstein *et al.* 2009).

Visible light photocatalysis has tremendous potential as a sustainable water treatment process, with the degradation pathways followed in the process being yet unclear. In this study an explanation on the photocatalytic degradation mechanism followed under visible light using TiO<sub>2</sub> is given. In this frame, identification and comparison of the intermediate products formed during photocatalysis under visible and UV-A light, using LC-MS/MS, together with Electron Paramagnetic Resonance (EPR) analysis has been performed.

## 2. Experimental

### 2.1. Materials

Both doped (C-doped TiO<sub>2</sub>, Kronos vlp-7000) and undoped (Evonik P25) TiO<sub>2</sub> materials were used under UV and visible light.

### 2.2. Instrumentation

Visible light irradiation (400–700 nm) was provided by a photolysis apparatus equipped with a cut-off filter exhibiting a zero transmittance below 410 nm. MC-LR and CYN degradation was monitored by HPLC-UV with detection at 238 nm and 262 nm, respectively (A. Hiskia *et al.* 2013; T. Fotiou *et al.* 2015). Determination of GSM and MIB in irradiated water samples was performed by headspace solid phase microextraction (HS-SPME)/ gas chromatography-mass spectrometry (GC-MS) (T. Fotiou *et al.* 2015; T. Fotiou *et al.* 2014).

Identification of reaction intermediates of MC-LR and CYN was carried out using LC-MS/MS analysis in full scan mode (A. Hiskia *et al.* 2013).

Spin trapping agents 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), 5-tert-Butoxycarbonyl - 5- methyl -1- pyrroline- N- oxide (BMPO) and 5 - (Diethoxyphosphoryl) -5- methyl -1-pyrroline-N-oxide (DEPMPO), with determination of their superoxide and the hydroxyl adduct. Also 2,2,6,6-tetramethylpiperidine (TEMP) was used for the detection of singlet oxygen.

### 3. Results

#### 3.1. Photocatalytic degradation of MC-LR, CYN, GSM and MIB under UV-A and visible light. Intermediate products of MC-LR and CYN under visible light

All four target compounds were studied for degradation under UV-A and visible light using Evonik P25 and Kronos vlp-7000 TiO<sub>2</sub>, commercially available photocatalysts. Under UV-A light all target compounds are completely degraded with both of the photocatalysts. With employment of visible light MC-LR and CYN were slowly degraded in the presence of Kronos only, whereas for GSM and MIB no degradation was observed.

In his study, identification of intermediate products formed during the photocatalytic degradation of MC-LR using Kronos vlp-7000 under visible light was performed using LC-MS/MS. A number of intermediate products were detected, with the products with the most intense peak areas being the *m/z* 795, 835, 1011.5 and 1029, (Table 1). Product with *m/z* 1029 is a dihydroxylation product on the conjugated diene structure system, of the Adda chain [Adda(OH)<sub>2</sub>] (A. Hiskia *et al.* 2013; Antoniou *et al.* 2010; Antoniou *et al.* 2008; Song *et al.* 2006) and has the highest peak area of all. The appearance of multiple peaks for *m/z* 1029 suggests the presence of a number of isomers of dihydroxy-MC-LR (A. Hiskia *et al.* 2013; M. G. Antoniou *et al.* 2008). Upon comparison with intermediates formed during the photocatalytic degradation of MC-LR under UV-A light, it was found that: (a) the number of intermediates was lower and (b) the number of isomers was also smaller, under visible light. These observations suggested different mechanism for visible and UV-A light.

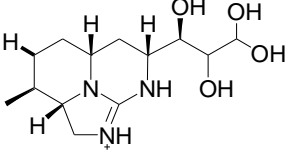
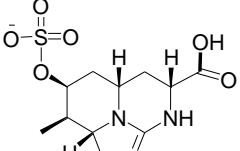
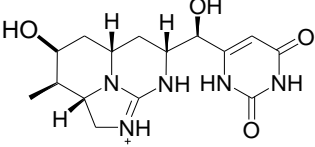
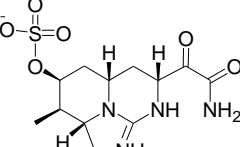
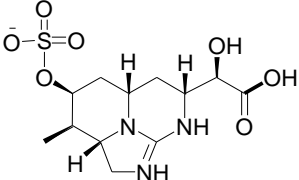
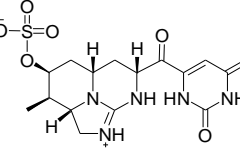
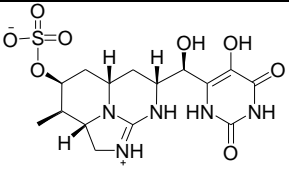
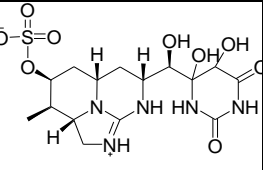
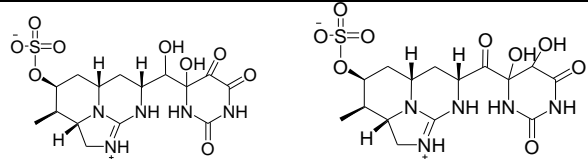
**Table 1:** Structures of reaction intermediates of MC-LR with TiO<sub>2</sub> based photocatalysts (Evonik P25 under UV-A and Kronos vlp-7000 under visible light)

No	Structure	<i>m/z</i>	Compound / MW
1	H-Arg-NHCH(OH)CH(CH <sub>3</sub> )CO-Glu-H	389	C <sub>15</sub> H <sub>28</sub> N <sub>6</sub> O <sub>6</sub> , 388.2
2	Arg-MeAsp-Leu-Ala-COOH	515.5	C <sub>21</sub> H <sub>37</sub> N <sub>7</sub> O <sub>8</sub> , 515.3
3	Arg-MeAsp-Leu-Ala-COCHOHCH <sub>3</sub>	544	C <sub>23</sub> H <sub>41</sub> N <sub>7</sub> O <sub>8</sub> , 543.3
4	Cyclo[-NHCH(CHO)CH(CH <sub>3</sub> )CO-Glu-Mdha-Ala-Leu-MeAsp-Arg-]	795	C <sub>34</sub> H <sub>54</sub> N <sub>10</sub> O <sub>12</sub> , 794.4
5	Cyclo[-NHCH(CHCHCOCH <sub>3</sub> )CH(CH <sub>3</sub> )CO-Glu-Mdha-Ala-Leu-MeAsp-Arg-]	835.5	C <sub>37</sub> H <sub>58</sub> N <sub>10</sub> O <sub>12</sub> , 834.4
6	(OH)-Cyclo[-Adda-Glu-Mdha-Ala-Leu-MeAsp-Arg-] Cyclo[-Adda(O)-Glu-Mdha-Ala-Leu-MeAsp-Arg-]	1011.5	C <sub>49</sub> H <sub>74</sub> N <sub>10</sub> O <sub>13</sub> , 1010.5
7	(OH) <sub>2</sub> -Cyclo[-Adda-Glu-Mdha-Ala-Leu-MeAsp-Arg-]	1027.5	C <sub>49</sub> H <sub>74</sub> N <sub>10</sub> O <sub>14</sub> , 1026.5
8	Cyclo[-Adda(OH) <sub>2</sub> -Glu-Mdha-Ala-Leu-MeAsp-Arg-] Cyclo[-Adda-Glu-Mdha(OH) <sub>2</sub> -Ala-Leu-MeAsp-Arg-]	1029	C <sub>49</sub> H <sub>76</sub> N <sub>10</sub> O <sub>14</sub> , 1028.6

In the case of CYN degradation intermediate products were also identified, some of previously found in a study with UV-A light (Table 2), (T. Fotiou *et al.* 2015). Among these, the main products formed were *m/z* 432, 448 and 450. Under visible light, product of *m/z* 448 has the highest peak area being the main intermediate and is supposed to be a di-hydroxylation product of CYN. It can be formed through reaction with HO<sub>2</sub>• on CYN's double bond, or after two HO• additions (T. Fotiou *et al.* 2015).

Results for CYN come in agreement with those in the case of MC-LR. In the relative abundance chromatograms of product *m/z* 448, two isomers were detected under UV-A light and only one isomer under visible irradiation.

**Table 2:** Structures of reaction intermediates of CYN with TiO<sub>2</sub> based photocatalysts (Evonik P25 under UV-A and Kronos vlp-7000 under visible light)

No	Structure	Compound / MW (m/z)	No	Structure	Compound / MW (m/z)
1		C <sub>13</sub> H <sub>24</sub> N <sub>3</sub> O <sub>4</sub> <sup>+</sup> , 286.18 (287)	2		C <sub>11</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S, 319.08 (320)
3		C <sub>15</sub> H <sub>23</sub> N <sub>5</sub> O <sub>4</sub> <sup>+</sup> , 337.17 (338)	4		C <sub>12</sub> H <sub>18</sub> N <sub>4</sub> O <sub>6</sub> S, 346.09 (347)
5		C <sub>12</sub> H <sub>19</sub> N <sub>3</sub> O <sub>7</sub> S, 349.09 (350)	6		C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>7</sub> S, 413.10 (414)
7		C <sub>15</sub> H <sub>21</sub> N <sub>5</sub> O <sub>8</sub> S, 431.11 (432)	8		C <sub>15</sub> H <sub>23</sub> N <sub>5</sub> O <sub>9</sub> S, 449.12 (450)
8					C <sub>15</sub> H <sub>21</sub> N <sub>5</sub> O <sub>9</sub> S, 447.11 (448)

### 3.2. ROS identification with EPR spin trapping

In order to identify the main ROS formed during the process, EPR analysis was employed. The approach used was through the spin trapping agents 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), 5-tert-Butoxycarbonyl - 5- methyl -1- pyrroline- N- oxide (BMPO) and 5 - (Diethoxyphosphoryl) - 5- methyl -1-pyrroline-N-oxide (DEPMPO), with determination of their superoxide and the hydroxyl adduct. Spin trapping studies were also conducted using 2,2,6,6-tetramethylpiperidine (TEMP) in order to investigate singlet oxygen's contribution in the process (Jia *et al.* 2014).

EPR analysis showed that during UV-A illumination process both hydroxyl and superoxide radical are produced, whereas under visible light illumination superoxide radicals and singlet oxygen is formed.

### 4. Conclusions

The main objective was the elucidation of the mechanism followed during the photocatalytic process under visible light irradiation. MC-LR and CYN were slowly degraded, while for GSM and MIB no degradation was observed under visible light. Identification and comparison of intermediates under both visible and UV-A light has been performed. Also EPR analysis for the identification of ROS species was employed with spin trapping techniques. Results showed that during visible light photocatalysis the main ROS is superoxide radical, whereas during UV light photocatalysis both hydroxyl and superoxide radicals were present.

## ACKNOWLEDGMENTS

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