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Processes influencing the destruction of microcystin-LR by TiO_2 photocatalysis

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Abstract

We have previously reported the effectiveness of TiO_2 photocatalysis in the destruction of the cyanotoxin microcystin-LR [P.K.J. Robertson, L.A. Lawton, B. Münch, J. Rouzade, J. Chem. Soc., Chem. Commun., 4 (1997) 393; P.K.J. Robertson, L.A. Lawton, B. Münch, B.J.P.A. Cornish, J. Adv. Oxid. Technol., in press]. In this paper we report an investigation of factors which influence the rate of the toxin destruction at the catalyst surface. A primary kinetic isotope effect of approximately 3 was observed when the destruction was performed in a heavy water solvent. Hydroxylated compounds were observed as products of the destruction process. No destruction was observed when the process was investigated under a nitrogen atmosphere.

Keywords: Microcystin-LR; Photocatalysis; Solvent

1. Introduction

Microcystin-LR is a hepatoxic material produced by several cyanobacteria general including *Microcystis*, *Anabaena* and *Planktothrix* [3]. This compound is a cyclic heptapeptide containing the amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), with leucine (L) and arginine (R) in the variable positions (Fig. 1). Microcystins have caused the deaths of both animals and humans as a result of ingestion of contaminated water [3–5]. It is also believed that longer-term exposure to sublethal levels of microcystins may promote primary liver cancer by disruption of protein phosphatases 1 and 2A. These enzymes are involved in regulating cell division particularly checking cell division [3].

Microcystins are chemically very stable [6] and conventional water-treatment processes have so far failed to remove them, furthermore the use of more advanced methods such as granular carbon filtration and photochemical degradation have shown only limited efficacy [7,8]. We have previously reported the effectiveness of TiO_2 photocatalysis in the destruction of microcystin-LR [1,2]. In this paper we have extended the investigation to examine the mechanism of this destruction at the photocatalyst surface. The results would indicate that the process occurs by attack of hydroxyl radical generated on the surface of the photocatalyst.

2. Experimental details

2.1. Materials

Microcystin-LR was purified from a natural sample of *Microcystis aeruginosa* [9]. The cyanobacterial cells were extracted in methanol followed by C18 reverse-phase flash chromatography (Biotage UK). The final purification was performed by preparative C18 reverse-phase chromatography.

Titanium dioxide (Degussa P-25) was used as received.

2.2. Photocatalysis

Aqueous solutions of microcystin-LR (reaction at pH 4 and temperature 306 K) were illuminated in the presence of air and a TiO_2 catalyst (1% m/v slurry of TiO_2 Degussa P-25) using a xenon UV lamp (280 W UVASpot 400 Lamp, Uvalight Technology, Spectral Output 330–450 nm). The same procedure was adopted for the experiments performed in D_2O.

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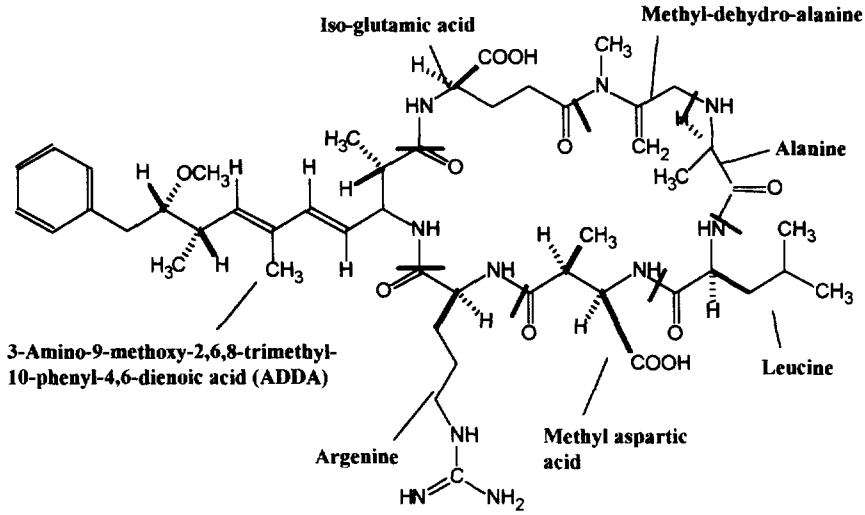

Fig. 1. Microcystin-LR (formula weight 994).

2.3. Analysis

The destruction of the samples was monitored by HPLC with a high resolution diode array detector (Waters 996 detector). Separations were performed on a symmetry C18 column (25 cm×0.46 cm I.D.; 5 mm particle size, Waters). Eluents were Milli Q water (Millipore, Watford, UK) and acetonitrile (Rathburn, Walkerburn, UK), both containing 0.05% trifluoroacetic acid (TFA). A linear gradient was employed starting at 30% (v/v) aqueous acetonitrile increasing to 35% over 5 min followed by an increase to 70% over the next 30 min. Detector resolution was set at 1.2 nm and the data acquired from 200 to 300 nm [10].

Mass spectrometry was performed using a Finnigan Masslab Navigator, with electrospray ionisation. This instrument utilises a quadrupole mass filter enabling measurement up to 1600 m/z.

3. Results

The destruction of microcystin-LR was investigated in both water (H_2O) and heavy water solvents (D_2O) (Fig. 2). The rate of toxin destruction in the D_2O solvent was significantly slower than that observed in water. The primary isotope effect (k_H/k_D) for the process was calculated to be 3 (Table 1). The intermediates of the destruction were investigated using mass spectrometry. Data from this analysis indicated that hydroxylated structures were generated (Fig. 3). The initial reaction appears to be the dihydroxylation of one of the double bonds of microcystin, witnessed by the increase from 995.7 m/z $[M+H]^+$ to 1029.7 m/z $[(M+2OH)+H]^+$. At the same time 835.5 m/z $[M+H]^+$ becomes prominent, and this is consistent with cleavage occurring at Adda CH=CMe. Also at this time a smaller peak at 817.5 m/z appears and this is consistent with the cleavage occurring at the Adda CH=CH $[M+Na]^+$. After a further 10 min the peak at 835.5 m/z disappears and a peak at 817.5 m/z becomes dominant. The proposed mechanism is then that dihydroxylation occurs at either of the Adda double bonds, followed by cleavage. The reason that the 835.5 m/z disappears altogether can be put down to the fact that a further cleavage step may occur at Adda CH=CH giving the 817.5 m/z fragment. This is a very stable product as is witnessed by the fact that it is present up to 100 min. Future studies are aimed at delineating the exact nature of the mechanism by high resolution mass spectroscopy and isolating the products using HPLC and identifying them using a combination of MS and NMR. In the case of the D_2O reaction OD groups attached to the molecule (Fig. 3). The rapid loss of toxicity of the toxin during the course of the photocatalytic treatment would also be consistent with an attack on this bond [2].

When the destruction was investigated under a nitrogen atmosphere no significant disappearance of the toxin was observed (Fig. 4).

4. Discussion

The role of the hydroxyl radical as the main oxidant in the photocatalytic destruction of organic compounds has long been suspected [11–14]. This concept has been validated for a wide range of compounds with evidence such as the demonstration of kinetic isotope effects [11], the formation of hydroxylated intermediates [12] and the detection of $OH^•$ in the reactor solutions [13]. A key paper by Turchi and Ollis [14] has reviewed the evidence that supports this hypothesis. This evidence included the observation of hydroxylated products of the process, the requirement for the catalyst to be hydroxylated for degradation to occur, the detection of $OH^•$ by ESR and kinetic isotope effects.

The results for the destruction of microcystin-LR would initially appear to be consistent with a hydroxyl radical attack on the toxin molecule. This supposition is reinforced by two factors:

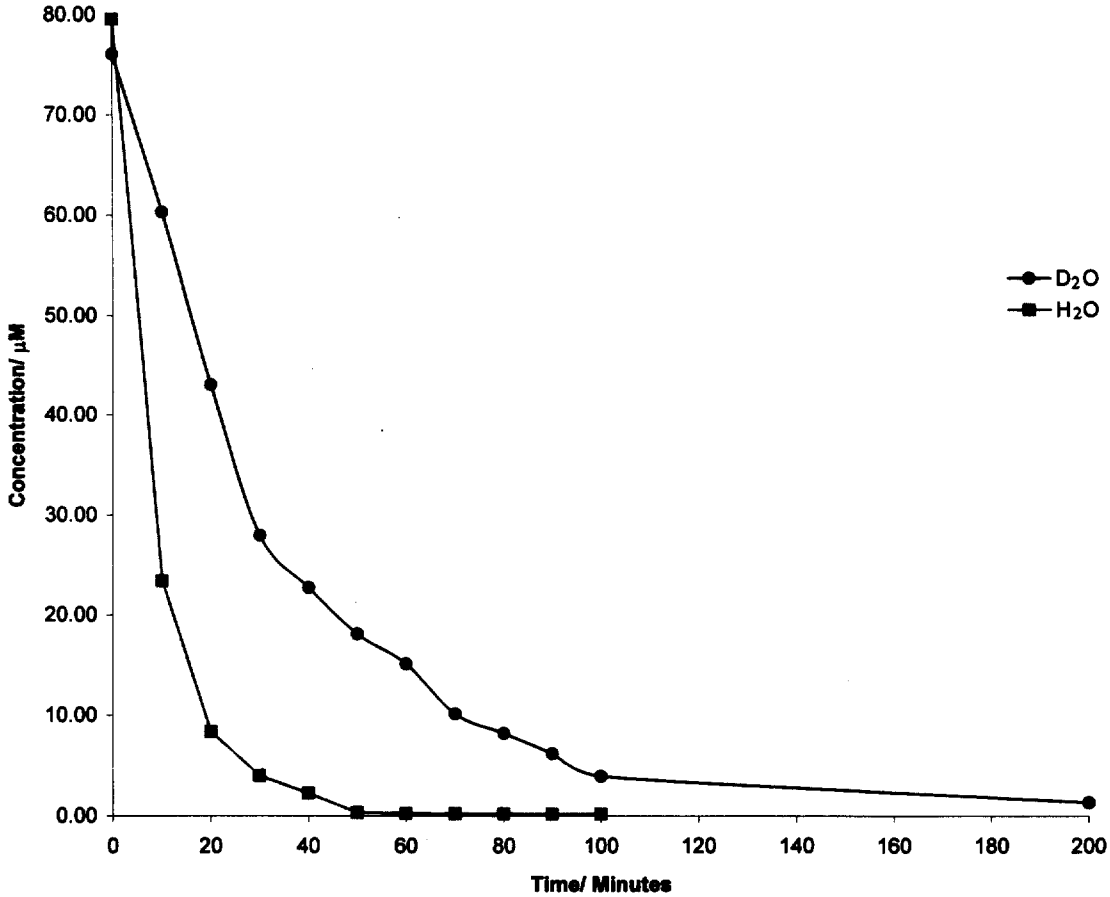

Fig. 2. The photocatalytic destruction of microcystin-LR in H_2O and D_2O solvents.

Table 1
Isotope effect based on rate of photocatalytic destruction of microcystin-LR

Solvent	Initial rate ($\mu M\ min^{-1}$)	Apparent first order rate constant (min^{-1})	Relative rate
H_2O	5.62	0.088	1.0
D_2O	1.58	0.029	0.33

1. the kinetic isotope effect observed in the D_2O solvent,
2. the detection of hydroxylated products from the photocatalytic destruction process.

Cunningham and Srijaranai [11] observed a similar kinetic isotope effect for the destruction of isopropanol using TiO_2 to that reported here. The results of both their work and ours suggest that a rate limiting process in the photocatalytic system is the formation of the hydroxyl species. The reduced rate in D_2O is due to the lower quantum efficiency for the

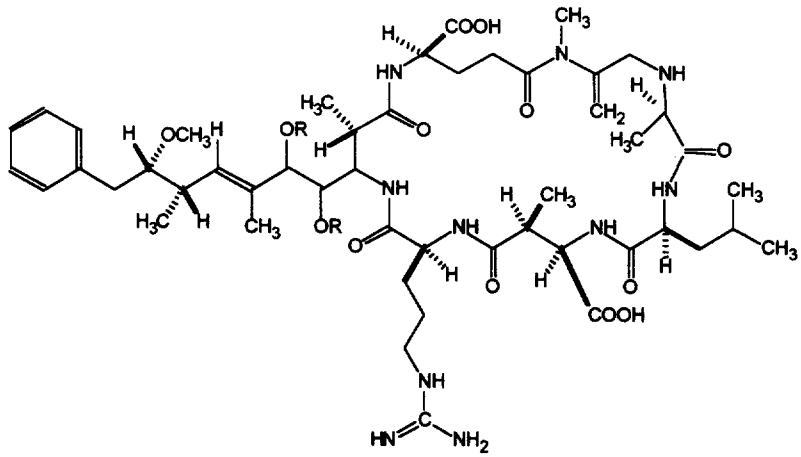

Fig. 3. The initial product of the photocatalytic destruction of microcystin-LR. R = H or D.

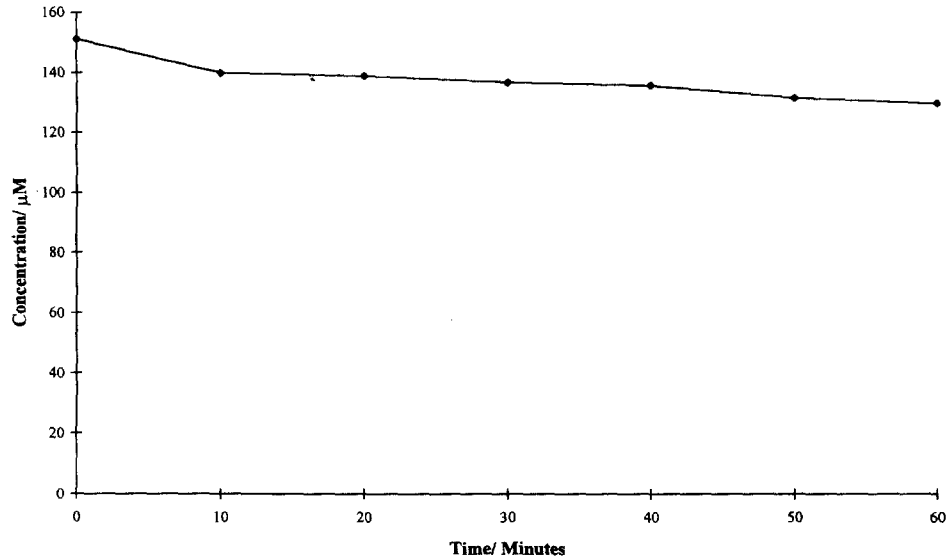

Fig. 4. The photocatalytic destruction of microcystin-LR under nitrogen atmosphere.

formation of OD$^{\bullet}$ radicals on the TiO_2 surface. Consequently there is a lower relative concentration of OD$^{\bullet}$ radical on the TiO_2 surface available for oxidation of the substrate. They proposed that this effect strengthened the supposition that the photogeneration of hydroxyl radicals was the rate determining process for the photocatalytic process. Few other workers have, however investigated the kinetic isotope effect with their particular systems.

Ollis and Turchi [14] established that using the same conditions the rate of destruction is the same for a variety of different organic compounds. They proposed that the rate destruction would depend on a variety of parameters including catalyst structure and illumination intensity. In addition they noted that the method for determining the rate constant for the process form the intercept of a double reciprocal plot was sensitive to small variations in the data. It is interesting to note that the rates observed by various groups for a diverse range of compounds are of a similar order [15–18].

Subsequent to the analysis by Ollis, it was proposed by Gerischer and Heller [19] and Wang et al. [20] that the rate-determining step for the destruction of organics by TiO_2 photocatalysis is the reduction of oxygen to superoxide radical anion. This observation confirmed that the rate of photocatalytic destruction would be independent of the substrate undergoing treatment since these compounds are not involved in the rate determining reaction.

In our investigation of microcystin we have also established that oxygen must be present for destruction of the toxin. This would indicate that neither microcystin nor any of the intermediates of destruction act as alternative electron acceptors. The level of oxygen required for this was in fact very small, with even pre-adsorbed gas allowing a limited destruction to proceed. Several other workers have also found the necessity of the presence of oxygen for photocatalytic processes [21–24].

Okamoto et al. [25] and Anpo et al. [26] have both proposed that the species formed as a result of the conductance band electron transfer to oxygen ($O_2^{\bullet -}$, $HO_2^{\bullet}$ and H_2O_2) are also involved in the photooxidation reactions. Lu et al. [21] established such a mechanism for the destruction of chloromethane on TiO_2. Using $^{18}O_2$ they established that oxidation was initiated via species generated from valence band reduction.

The precise nature of the oxidising agent involved in the photocatalytic process is therefore, still a matter of debate. Product analysis may be inappropriate for assigning a particular mechanism. The presence of the hydroxylated products may not necessarily confirm attack by OH$^{\bullet}$ radicals. The substrates may undergo attack by direct hole oxidation and then subsequently may be hydrated by the solvent [27–29]. In order to determine the most likely primary oxidation process there is therefore a requirement for some other experimental evidence.

The fact that the kinetic isotope effect observed by us was of a similar magnitude to that observed by Cunningham and Srijaranai [11] may be significant. It is possible that the destruction of the toxin is mediated via hydroxyl radicals generated form the superoxide radical anion produced at the conduction band. This is subsequently hydrated or deuterated by the solvent. This may be rate determining since the O_2 has to be generated at the conduction band prior to interaction with the solvent and subsequent formation of OH$^{\bullet}$ or OD$^{\bullet}$ species. Therefore the kinetic isotope effect could be due to the interaction of the solvent with the superoxide species rather than the attack on the toxin. If this is the case we should observe a similar kinetic isotope effect no matter what the substrate being treated.

For the oxidation of microcystin to proceed there is therefore a requirement for hydroxyl radicals and oxygen. Whether the rate-determining step for the process is the degeneration

of the hydroxyl radicals or the reduction of oxygen has yet to be determined.

5. Conclusion

The destruction of microcystin-LR appears to be initiated via hydroxyl radical attack on the ADDA group of the toxin. This premise is based on the fact that a kinetic isotope effect of 3 was observed when the destruction was investigated in D_2O. In addition hydroxylated compounds were observed as products of the decomposition process. Toxicity testing of solutions treated by this method have confirmed that the by-products of the photocatalytic process are nontoxic [2].

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