Processes influencing surface interaction and photocatalytic destruction of microcystins on titanium dioxide photocatalysts.

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Abstract

Microcystins are a family of hepatotoxic peptides produced by freshwater cyanobacteria. Their occurrence in drinking water is of concern since chronic exposure to these toxins causes tumour promotion. It is therefore essential to establish a reliable treatment strategy that will ensure their removal from potable water. We have previously described the rapid destruction of microcystin-LR using TiO_2 photocatalysis, however, since there are at least 70 microcystin variants it is essential that the destruction of a number of microcystins be evaluated. In this study the dark adsorption and destruction of four microcystins was followed over a range of pH. All four microcystins were destroyed although the efficiency of their removal varied. The two more hydrophobic microcystins (-LW and -LF) were found to have high dark adsorption (98 and 91% at pH 4) in contrast to microcystin-RR, which was found to have almost no (only 2-3%) dark adsorption across all pH.

Keywords: TiO_2; Photocatalysis; Surface Adsorption; Microcystins; Cyanotoxins; Structure-degradation relationship

INTRODUCTION

Microcystins are a group of at least 70 hepatotoxic peptides produced primarily by freshwater cyanobacteria (blue-green algae) belonging to the genera *Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria* (*Planktothrix*). Their presence in water bodies has caused illness and death of wild and domestic animals world-wide (1), and in 1996 they caused human fatalities due to their presence in dialysis water (2). Their potential for causing both acute and chronic toxicity has increased pressure to ensure their removal from potable water, and in 1999 WHO published a guideline value for microcystin-LR of 1 μg L^{-1} (3).

Microcystins are cyclic heptapeptides that share a general structure (Fig. 1) containing γ-linked D-glutamic acid (D-Glu), D-alanine (D-Ala), *β*-linked D-*erythro*-*β*-methylaspartic acid (D-MeAsp), *N*-methyldehydroalanine (Mdha) and a unique C_{20} *β*-amino acid, (2*S*, 3*S*, 8*S*, 9*S*)-3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid (Adda). The other two L-amino acids are variable (denoted **X** and **Z)** and found in position 2 and 4 of the cyclic structure. The single letter abbreviation of the variable amino acids is used to distinguish different microcystins; for example, the most commonly occurring microcystin contains leucine (L) and arginine (R) in these positions and is therefore called microcystin-LR. Variation in these two amino acids accounts for many of the microcystin variants that have been characterised but other minor modifications such as demethylation increases the number of microcystin variants to at least 70 (1).

The removal and/or destruction of microcystins in drinking water has been evaluated for most of the commonly used treatment strategies and while some chemicals (e.g. chlorine and ozone) have been used with some degree of success there is a requirement for a routine reliable method (4). The use of TiO_2 photocatalysis has

been established as a useful technique for the destruction of a wide range of organic pollutants (5), and we have previously reported the effectiveness of this technique in the destruction of microcystin-LR (6,7). However, since a wide number of microcystin variants are produced in nature and may be present in drinking water sources it is essential to evaluate a range of different microcystins. The TiO_2 photocatalysis of microcystin-LR, -RR, -LW and -LF was studied to determine the influence of a single amino acid substitution on both the initial rate of destruction and dark adsorption. Furthermore, the influence of pH was also assessed to determine the most suitable conditions for drinking water treatment.

EXPERIMENTAL METHOD

Chemicals

Microcystins (-LR, -RR, -LW and -LF) were purified from laboratory cultures of *Microcystis aeruginosa* using methods previously described by Edwards et al. (8) and Lawton et al. (9). Microcystin solutions were prepared in Milli-Q grade water (Millipore, Watford, U.K.). Titanium Dioxide (P-25, Degussa, U.K.) was used as received.

Photocatalysis

Aqueous solutions (10 ml) of individual microcystins (unaltered pH was 4 and temperature 306 K) were illuminated in the presence of air and TiO_2 (1% w/v) using a xenon UV lamp (480 W UVASpot 400 lamp, Uvalight technology Ltd with spectral output 330-450 nm). The photonic output of the lamp was determined to be 2.48×10^{-5} einstein min^{-1} using ferrioxalate actinometry. Reactions were carried out in thick-walled glass universal bottles with constant stirring and pH of the starting solution adjusted using either nitric acid or sodium hydroxide. Initial pH of 1, 4, 5, 7, 10 and 12 were used to explore the influence of pH on the reaction. Samples were removed prior to catalyst addition and at timed intervals during the reaction. Microcystin solutions in the absence of catalyst were also prepared at each test pH to determine the affect of pH alone on microcystin stability. Microcystin concentrations (100 – 200 $\mu g\ ml^{-1}$) were selected to allow direct analysis by HPLC.

Analytical methods

Analysis of microcystins was performed by HPLC with photodiode array detection as previously described by Lawton et al. (10) with the following

modifications: column, Symmetry C18 250 x 4.6 mm i.d.(Waters, Watford, UK); detection, 996 diode array (Waters) monitoring between 200 and 300 nm (resolution, 1.2 nm). All chromatograms were analysed at 238 nm.

Calculation of net charge

To interpret the factors influencing the interactions between the catalyst and different microcystins, it is necessary to identify the likely species, protonated or dissociated, which may occur in the different pH ranges. Thus, for microcystin-RR, with two carboxylate groups (Asp, MeAsp) and two guanidino groups (Arg), we would have H_4A^{2+}, H_2A, and finally A^{2-} as pH increases. This is assuming that the pairs of similar groups are independent of each other, and will dissociate simultaneously. The following pKa values were used (11): Asp and MeAsp COOH pKa 2.10; Arg guanidino group pKa 12.48. The indole NH of Trp has pKa values of -2.4 and 16.97, and is therefore not expected to show any degree of protonation or deprotonation in the range pH 0-14.

Calculation of the fraction of each species present ($f_{species}$), relative to the total concentration of the molecule, as a function of the pH was the performed. This was achieved by first calculating the inverse fraction - the Ringbom side reaction coefficient ($\alpha_{species}$) - and then taking the reciprocal (12). K_{a1} and K_{a2} are the acid dissociation constants for the first and second protons.

$$1/f_A = \alpha_{A(H)} = \frac{[H_4A^{2+}]+[H_2A]+[A^{2-}]}{[A^{2-}]} = \frac{[H^+]^2}{K_{a2}.K_{a1}} + \frac{[H^+]}{K_{a2}} + 1 \qquad [1]$$

Similar equations can be used for each of the different protonated species. The net overall charge is calculated by summing the fraction multiplied by the charge:

Net charge = f_{H4A}. (2+) + f_{H2A}. (0) + f_A. (2-) [2]

RESULTS AND DISCUSSION

The photocatalytic destruction of four microcystins was investigated using a TiO_2 photocatalyst. In each case it was found that they could all be successfully destroyed in the presence of TiO_2 and UV light (Table 1). No destruction was observed in any control samples. Dark adsorption and rate of toxin destruction were found to be influenced both by pH and the amino acid composition of the microcystins (Table 1 and 2). Microcystins all possess the same generic structure (Fig. 1) with two of the seven amino acids being variable (position 2 and 4). Three of the microcystins used in this study contain leucine in position two with the amino acid present in position 4 conferring changes in the hydrophobicity of the overall molecule. The effect on the dark adsorption of this single amino acid substitution is clearly observable (Table 2). Approximately half of the microcystin-LR was found to adsorb to the surface of the TiO_2 prior to UV illumination under typical reaction conditions (pH 4). The extent of adsorption, however, increased to over 90% for the two microcystin variants that contained a hydrophobic amino acid in position 4, i.e., microcystin-LF and microcystin-LW. In contrast, the more polar microcystin-RR, a variant that contains arginine in both variable positions demonstrated only 2 % dark adsorption at pH 4.

We have previously shown that change in pH influenced both dark adsorption and the initial rate of destruction for microcystin-LR (13). This corresponds to the changes in microcystin hydrophobicity, which was reported to increase as pH decreases (14). This work demonstrated that at pH values of 2 or below a D_{ow} (octanol/water

distribution ratio) of approximately 2 was observed. Subsequently, as the pH increased the observed D_{ow} decreased to below -1 representing a change in hydrophobicity by three orders of magnitude (14). It can clearly be seen that, like microcystin-LR, the dark adsorption of both microcystin-LW and -LF were strongly influenced by solution pH. Microcystin-LF demonstrated similar characteristics to microcystin-LR, with a maximum adsorption at pH 4. Microcystin-LW, however, demonstrated very high dark adsorption between pH 1 to 7. The dark adsorption of microcystin-RR changed little with modification of solution pH with most (>95%) of the microcystin-RR remaining in solution at each pH investigated. All the microcystins used in this investigation demonstrated very low or no adsorption under basic conditions (pH 10 and 12).

These findings clearly demonstrate that hydrophobicity is a contributory factor influencing the initial adsorption of microcystins to the surface of TiO_2. Through the hydrophobic effect, hydrophobic compounds preferentially move to surfaces from the bulk of aqueous solvents (5). In the case of charged organic compounds surface adsorption may be due to both hydrophobic and electrostatic effects (5).

Pichat et al. (15) previously reported an increase in apparent first order rate constant with increasing hydrophobicity of a series of chlorophenols. In a follow up study of meta and para substituted methoxybenzenes no simple correlation was observed between the hydrophobicity (K_{ow}) of the compound and the first order rate constant for the photocatalytic decomposition process (16). They proposed this discrepancy was possibly due to an interaction between adsorbate molecules resulting from van der Waals forces. In both cases reported by Pichat (15,16), however, the

effect of pH was not investigated and the pH of the system under investigation was not reported. The rate of photocatalytic reactions of some species on TiO_2 photocatalysts has been found to be significantly influenced by pH. The point of zero charge (pH_{zpc}) of TiO_2 is around pH 6.25 (5); below this level the surface is positively charged while above pH 6.25 TiO_2 is negatively charged. Therefore if pH> pH_{zpc} the oxidation of cationic electron donors and acceptors should be favoured while the destruction of anionic electron donors and acceptors should be preferred if pH< pH_{zpc} (5). In studies of the photocatalytic oxidation of trichloroacetic acid and secondary amines on TiO_2 Kormann et al. (17) noted differing pH effects for the destruction of both species. With trichloroacetic acid an increase in oxidation rate with decreasing pH below the pH_{zpc}. It was proposed that chloroacetic acid was involved in innersphere surface complexation with surface sites of TiO_2 at lower pH levels. However, pH levels above the pH_{zpc} appeared to favour the oxidation of protonated secondary amines. At these pH levels surface complexation of this species is more likely (5, 17).

A calculation of the net charge at varying pH values shows the different behaviour for the different microcystins (Fig. 2). Since the charge bearing groups on microcystin-LF and -LW are the same within the range pH 1-14, their curves are expected to be the same. The effect of the net charge on the microcystins should therefore also influence dark adsorption. Microcystin-LW and -LF bear negative charge over the whole pH range (Fig. 2), and are therefore expected to adsorb to the positively charged surface of the catalyst below pH 6.25 and to show reduced adsorption above this pH. It was indeed found that there was a large degree of dark adsorption of both microcystin-LW and-LF below pH 7, and almost none above this

value (Table 2). It was observed that microcystin-LW matches this predicted pattern exactly, however, the adsorption of microcystin-LF greatly reduced at pH of 5 and above. Since both microcystins are the same except for the exchange of the indole group in microcystin-LW for the phenyl group in microcystin-LF, the differing pH adsorption effects must be due to these structural effects. The hydrophobicity of tryptophan is much greater (2.25) than that of phenylalanine (1.79). It can therefore be proposed that the difference in the degree of adsorption may be due to the difference in hydrophobic interactions between microcystin-LW and -LF, and the TiO_2 surface.

Microcystin-LR is positively charged below pH 2.10 and negatively charged above this point. We therefore expect to see significant dark adsorption between pH 2.10 and 6.25, above pH 6.25 the catalyst becomes negatively charged and will repel microcystin-LR. This is manifested in the experimental results, which indicate that there is a large degree of dark adsorption between pH 4 and 7 (Table 2). Microcystin-RR is positively charged between pH 2 and 4, and is therefore not expected to adsorb to the positively charged surface of the photocatalyst. Above pH 10, microcystin-RR is negatively charged and is not expected to adsorb to the negatively charged surface. The lack of dark adsorption between pH 4 and 10, when microcystin-RR is charge-neutral, may be due to the high solubility of microcystin-RR in water. Alternatively, the low hydrophobicity of arginine (-1.01) may result in very poor hydrophobic interactions between microcystin-RR and the TiO_2 surface. These findings therefore suggest that, in this catalyst system, dark adsorption is charge-mediated, although the hydrophobicity of the amino-acid side chains also plays a role.

A relationship between initial reaction rate and dark adsorption has also been

observed for the photocatalytic destruction of microcystin-LR (13), suggesting that for efficient removal of the toxin good initial adsorption is necessary. This also suggests that the photocatalytic oxidation is taking place on or near the surface of the catalyst. Our findings here further support this in that the most rapid removal occurs when high dark adsorption was observed (Table 2). When examining the relationship between percentage destruction (Table 1) and dark adsorption (Table 2) a clear correlation can be seen where the highest dark adsorption results in the greatest percentage destruction. Although preliminary examination of initial rate data conflicts with this observation (Table 3), it should be noted that in the case on microcystins-LW and -LF the level of dark adsorption is so great (>90%) this masks a true determination of an accurate initial rate.

Feitz *et al.* (18) also concluded that adsorption was necessary for rapid degradation with a maximum initial rate of degradation observed at pH 3.5 for microcystin-LR and this concurred with the highest dark adsorption. Only one other TiO_2 photocatalysis study (19) has investigated the successful destruction of several microcystin variants (microcystin-LR, -YR and -YA) however, neither dark adsorption or initial rates were reported therefore no comparison with the present study can be made.

CONCLUSION

The photocatalytic destruction of microcystins is clearly pH dependent. This pH dependence can be associated with both changes to surface charge of the photocatalyst and altered hydrophobicity and net charge on the toxin. The different amino acid composition of the microcystin variants clearly influences the rate of

photocatalytic destruction through charge and hydrophobicity influences on the toxin molecule. Although dark adsorption of the toxin is desirable for photocatalytic decomposition it is not essential as demonstrated by the behaviour of microcystin-RR. When considering large-scale application of this process for removal of microcystins from potable waters, optimisation of reaction conditions must be performed with a range of representative microcystin variants.

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Figure Legend

FIG. 1 Generic structure of microcystins where X and Z represent the variable amino acids and, D-Me-Asp is D-*erythro*-*β*-methylaspartic acid, Adda is(2*S*, 3*S*, 8*S*, 9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid , D-Glu is D-glutamic acid and Mdha is *N*-Me-dehydroalanine.

FIG 2. Calculated net charges for microcystin-LF, -LR and -LW at varying pH values.

	% microcystins destroyed after (a) 10 min. and (b) 30 min							
	Microcystin-LR		Microcystin-RR		Microcystin-LW		Microcystin-LF	
pH	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
1	51	83	44	78	100*	100*	100*	100*
4	97	100*	53	83	100*	100*	100*	100*
5	81	100*	39	75	100*	100*	100*	100*
7	83	100*	14	37	100*	100*	100*	100*
10	11	58	56	71	58	100*	100*	100*
12	9	27	52	71	85	100*	98	100*

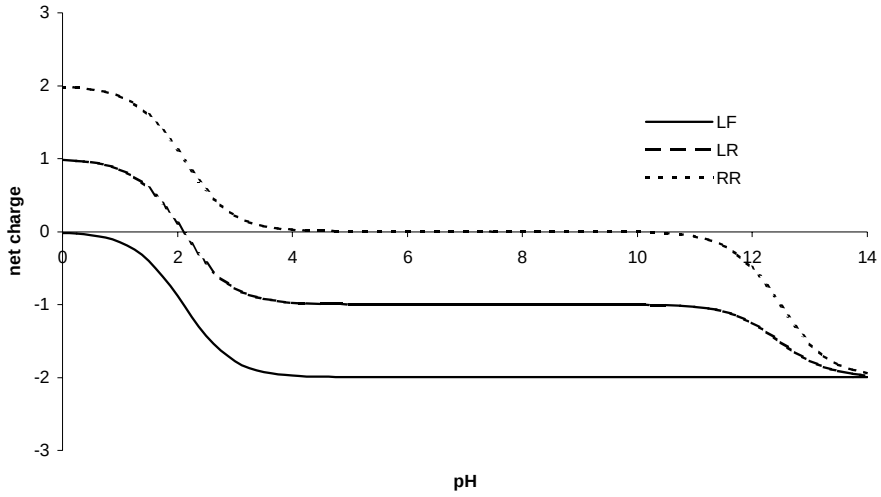
Table 1. Effectiveness of TiO_2 destruction of microcystin-LR, -RR, -LW and -LF after 10 and 30 minutes photocatalysis (*< 200 ng ml^{-1}).

	% Dark Adsorption			
pH	Microcystin-LR	Microcystin-RR	Microcystin-LW	Microcystin-LF
1	8	2	87	72
4	48	2	98	91
5	38	3	95	20
7	45	3	95	5
10	1	2	0	0
12	2	2	0	10

Table 2. Relationship between pH and TiO_2 dark adsorption of microcystin-LR, -RR, -LW and -LF.

	Initial Rate of Toxin Destruction/ μg ml^{-1} min^{-1}			
pH	Microcystin-LR	Microcystin-RR	Microcystin-LW	Microcystin-LF
1	12 (0.48)	10 (0.40)	1.7 (0.07)	7.3 (0.29)
4	33 (1.33)	10 (0.40)	0.4 (0.02)	3 (0.12)
5	13 (0.52)	5 (0.20)	0.4 (0.02)	25 (1.01)
7	15 (0.60)	2 (0.08)	0.4 (0.02)	27 (1.09)
10	1 (0.04)	10 (0.40)	10 (0.40)	23 (0.93)
12	5 (0.20)	10 (0.40)	18 (0.73)	20 (0.81)

Table 3. Relationship between reaction pH and the initial rate of TiO_2 photocatalytic destruction of microcystin-LR, -RR, -LW and -LF. Initial photonic efficiencies indicated in parenthesis.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