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Removal of microcystins from a waste stabilisation lagoon: Evaluation of a packed-bed continuous flow TiO_2 reactor

Carlos J. Pestana[a,b]*, Peter Hobson[a], Peter K. J. Robertson[c], Linda A. Lawton[b], Gayle Newcombe[a]

[a] Australian Water Quality Centre, South Australian Water Corporation, 250 Victoria Square, Adelaide, SA 5000, Australia

[b] School of Pharmacy and Life Sciences, Robert Gordon University, Sir Ian Wood Building, Garthdee Road, AB10 7GJ, Aberdeen, UK

[c] School of Chemistry and Chemical Engineering, Queen's University, David Keir Building, 39 Stranmillis Road, BT9 5 AG, Belfast, UK

*Corresponding author:

Email: c.pestana@rgu.ac.uk

Tel: 0044 1224 262847

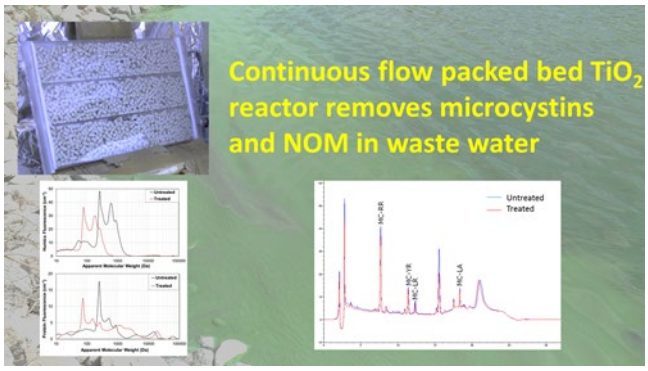
Declaration of Interest: None.

Highlights:

- Removal of microcystins from waste stabilisation lagoon
- Continuous flow, packed bed TiO_2 reactor design
- Significant removal of microcystins and improvement of water quality

- Natural organic matter decreases but does not inhibit microcystin removal
- Viable treatment option if optimised for toxin and cell removal

Graphical abstract:



Abstract

Photocatalysis has been shown to successfully remove microcystins (MC) in laboratory experiments. Most research to date has been performed under ideal conditions in pure or ultrapure water. In this investigation the efficiency of photocatalysis using titanium dioxide was examined in a complex matrix (waste stabilisation lagoon water). A flow-through photocatalytic reactor was used to photocatalyse four commonly occurring microcystin analogues (MC-YR, MC-RR, MC-LR, and MC-LA). Up to 51% removal for single MC analogues in waste lagoon water was observed. Similar removal rates were observed when a mixture of all four MC analogues was treated. Although treatment of MC-containing cyanobacterial cells of *Microcystis aeruginosa* resulted in no decline in cell numbers or viability with the current reactor design and treatment regime, the photocatalytic treatment did improve the overall quality of waste lagoon water. This study demonstrates that

despite the presence of natural organic matter the microcystins could be successfully degraded in a complex environmental matrix.

Keywords: waste water treatment; cyanobacteria; photocatalysis; titanium dioxide; microcystin

1. Introduction

Cyanobacteria commonly occur in waste stabilisation lagoons, usually applied to remove phosphates and nitrates, within waste water treatment plants (Barrington et al., 2013; Martins et al., 2011). Certain cyanobacterial strains are capable of producing toxic secondary metabolites; especially the microcystins (MC) are of rising worldwide concern and responsible for the intoxication of humans and animals (Ghadouani and Coggins, 2011; Paerl and Huisman, 2008). MC are hepatotoxic cyclic peptides with a wide variety of congeners (246 to date) (Spoof and Catherine, 2017); one of the most common and toxic congeners is MC-LR (LD_{50} in mice 50 μg kg^{-1}) (Sivonen and Jones, 1999). The World Health Organisation has published a recommended maximum allowable level for MC-LR of 1 μg L^{-1} in drinking water (WHO, 2017). While the waste lagoon effluent is not destined for human consumption, it can be used for irrigation of crops and recreational areas or discharged into receiving water bodies (Barrington et al., 2013). While the health and safety risk is comparatively low when compared to drinking water MCs can nonetheless pose significant ecological issues. Additionally cyanobacteria and/or their secondary metabolites are known to hinder waste water treatment processes (Martins et al., 2011; Praptiwi et al., 2017).

Chemical oxidation with hydroxyl radicals (OH·) generated by UV titanium dioxide (TiO_2) photocatalysis has previously been shown to successfully remove MC-LR and other congeners (Lawton and Robertson, 1999; Liu et al., 2002; Pestana, 2012; Pestana et al., 2015). However, there remain barriers to the application of this technology: the form of the catalytic material and subsequent separation of the catalyst from water, as well as the development of a continuous flow reactor to allow incorporation of the process in-line with treatment processes. Nano-particulate TiO_2 (e.g. Degussa P25) has a large reactive surface area (Lawton and Robertson, 1999; Robertson et al., 1997), however, separation from water is challenging and prevents ease of use. Pelletised TiO_2 facilitates separation from the sample matrix, however, effectiveness is reduced compared to nano-particulate TiO_2 (Liu et al., 2009). Coated surfaces have been demonstrated to be as effective as nano-particulates however, these require a specialised, energy demanding production process (Pelaez et al., 2010). Furthermore, previous studies (Lawton and Robertson, 1999; Liu et al., 2009, 2002; Pelaez et al., 2010; Robertson et al., 1997) evaluated removal of MC in pure water, whereas, in environmental applications other organic material would be present, competing with MC. Therefore, real-life applications will potentially experience decreasing effectiveness or alternatively requiring longer exposure times. Pestana et al. (2014) have recently successfully demonstrated the UV photocatalytic removal of the commonly problematic taste and odour compounds geosmin and 2-methylisoborneol with a packed-bed, continuous flow reactor using pelletised TiO_2 in water from a fish-farm raceway. This work demonstrated that a flow-through reactor design with pelletised TiO_2 is capable of removing trace water contaminants from a complex matrix.

In the present study, a bench scale packed-bed, continuous flow reactor was designed and used to demonstrate the UV photocatalysis of four commonly occurring MC congeners in waste lagoon water using pelletised TiO_2. Waste stabilisation lagoon water presents an extremely complex sample matrix with high dissolved organic material which present a challenge to the successful removal of toxic contaminants like MC, as natural organic matter (NOM) can exhibit radical scavenging properties and light attenuation. This represents an important step towards the implementation of the full-scale application of continuous-flow TiO_2/UV in water treatment applications.

2. Materials and methods

2.1 Chemicals

Hombikat K01/C titanium dioxide pellets (Supporting Information, S1) were obtained from Sachtleben Chemie (Germany) and rinsed thoroughly with reverse osmosis water (RO) before use for fines removal. Microcystin analogues were obtained from Enzo Life Science AG (Switzerland). All solvents were obtained from Merck (Germany) and were of analytical grade.

2.2 Photocatalysis of toxins

A packed-bed continuous flow reactor was constructed and connected to a 3 L reservoir. In brief, the reactor consisted of a three channel (connected in a serpentine fashion) sheet of polycarbonate with a guaranteed 95% UV permeability (Evonik, Germany) that was packed with pelletised TiO_2 (Sachtleben Chemie, Germany). Silicone tubing and a peristaltic pump recircled the test solution through the reactor (Supporting Information, S2). A test solution (autoclaved lagoon water or

RO filtered water) with 30 μg L^{-1} of the relevant microcystin analogue was prepared. In the case of the MC analogue mixture, 10 μg L^{-1} of each of the four analogues under investigation (MC-YR, -RR, -LR,-LA) were added. Initially, a T0 sample was taken, followed by the entire experimental volume of the reservoir passing through the reactor three times (contact time 1.9 min; see S2 for calculation of contact time) without irradiation to determine the dark absorption, i.e. the amount the MC concentration decreases in the absence of UV which is attributed to adsorption onto the TiO_2. The time was deemed sufficient based on the results of the controls that were performed in the dark and the absence of UV irradiation (Supporting Information, S3). Following this, the test solution was recycled through the illuminated reactor for a total illuminated contact time of 14.3 min with samples taken at known intervals.

2.3 Photocatalysis of *Microcystis aeruginosa*

A 17 day old culture of *Microcystis aeruginosa* 338 (Australian Water Quality Centre) was aseptically separated from growth medium by centrifugation (15 min, 1640 x *g* at room temperature). The cell pellets were re-suspended in waste lagoon water (100 mL). A sample was taken, fixed with Lugol's solution, and cell enumeration was performed using a Sedgewick-Rafter cell and light microscopy (Nikon 50i, Japan). The culture was analysed by flow cytometry using fluorescein diacetate (FDA) and SYTOX Green dyes to measure cell viability and membrane integrity using previously published protocols (Hobson et al., 2012). A cell suspension was prepared (3 x 10^5 alive cells mL^{-1}) in waste stabilisation lagoon water. The photocatalysis experiment was then performed as before (section 2.2) without the

addition of MC. Controls were performed in the absence of UV irradiation and in the absence of catalyst.

2.4 Analysis

Samples for MC analysis were concentrated by solid phase extraction (SPE) through C18 cartridges (Waters, United Kingdom) as previously described (Nicholson et al., 1994). After SPE the eluted samples were reduced to dryness by centrifugal evaporation in a MiVac DuoConcentrator attached to a MiVac DuoPump (both GeneVac, United Kingdom) and re-suspended in 50% aqueous methanol (1 mL), filtered (0.22 µm), and analysed by HPLC. The samples from the photocatalysis of the MC-containing *M. aeruginosa* were filtered (GF/C; Whatman, UK) to separate cells from the water. The filter disks containing the cells were frozen (-20 °C) then extracted in 10 mL of 80 % methanol for 60 min with occasional agitation (Lawton et al., 1994). The extract was reduced to dryness by centrifugal evaporation, re-suspended in 50 % methanol (1 mL), filtered (0.22 µm), and analysed. The MC in the cell filtrate was concentrated as before by SPE to determine extra-cellular MC concentration.

All processed samples were analysed on an Agilent Technologies 1100 series high performance liquid chromatography (HPLC) system consisting of a quaternary pump (G1311A), degasser (G1379A), auto sampler (G1313A), column compartment (G1316A) and photodiode array detector (G1315B) using the previously published method of Ho *et al*. (2006). The extraction recoveries for the above method are stated as > 95% (Ho et al., 2006a). Samples of treated and untreated waste lagoon water, along with the light and dark controls, were analysed by high performance size exclusion chromatography (HPSEC) for analysis of the effect the UV/TiO_2

treatment on the protein and humic portions of the NOM. For this, samples were analysed on a Waters Alliance 2690 separations module and 996 photodiode array detector (PDA) at 260 nm (Waters Corporation, USA) according to a method described in Fabris et al. (2008). A 0.1 M Phosphate buffer with 1.0 M NaCl was passed through a packed silica column (Shodex KW802.5; Showa Denko, Japan) at a flow rate of 1.0 mL min^{-1}. The effect of the treatments on water quality parameters was also monitored (Supporting Information S4).

3. Results and Discussion

3.1 Evaluation of reactor performance

Previously published studies of UV/TiO_2 removal have almost exclusively employed pure water, thus a comparison between the removal in pure water and waste lagoon water was performed. The matrices tested were RO water and waste lagoon water (see Supporting Information S4 for water quality parameters of untreated lagoon water). The removal of MC-LR dropped from 84% in RO water to 46% in waste lagoon water under the same experimental conditions (figure 1), with the most probable explanation being the presence of competing natural organic matter (NOM). Although another contributing factor could be the presence of inorganic ions that adhere to the TiO_2 and inactivate binding places by blocking them for organic molecules (Umar and Aziz, 2013), indicated by a decrease in conductivity from 1173 to 976 µS cm^{-1} (Supporting Information, S4). Furthermore, NOM can act as a scavenger of the surface-generated oxidising species (e.g. OH and superoxide radicals) produced by interaction of the catalyst and the UV irradiation in the aqueous matrix (Feitz et al., 1999). Pelaez et al. (2011) also found that NOM had an inhibitory effect on the photocatalysis of MC-LR when they investigated the

photocatalytic breakdown of MC-LR both in the presence of naturally occurring humic and fulvic acids.

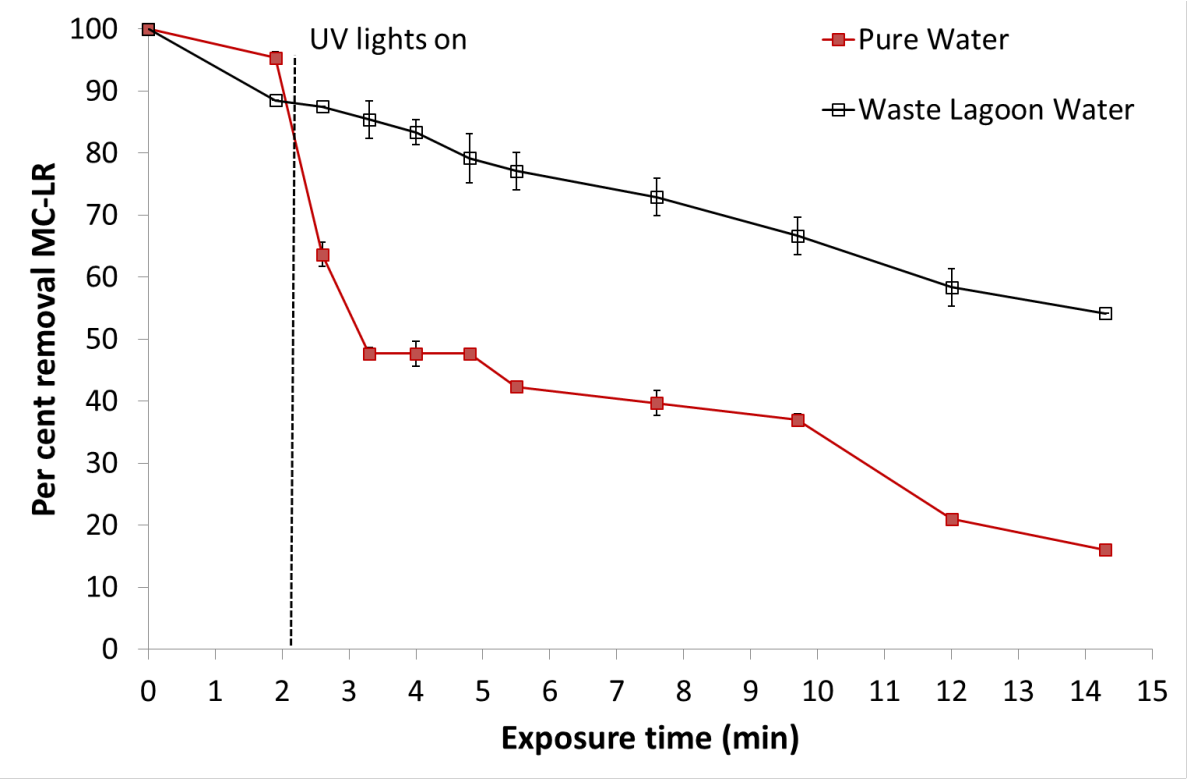


Figure 1: Removal of MC-LR (initial concentration 30 µg L^{-1}) in TiO_2/UV packed-bed flow-through reactor comparing efficiency in pure water (RO filtered water) - ■ and waste lagoon water - □. (Error bars=1 SD, *n*=2)

In the work of Pelaez et al. (2011) the adsorption of NOM to TiO_2 at different pH levels was investigated and it was found that the adsorption of NOM to the surface of the catalyst increased with decreasing pH, with highest adsorption rates observed under acidic conditions (pH 3.0). Other research has suggested that under acidic conditions another competing process could operate. Paul et al. (2007) suggested that the adsorption of fluoroquinolone (a synthetic antibiotic) to TiO_2 could lead to the formation of a surface coordination complex that could facilitate the transfer of electrons from the conduction band of TiO_2 to an appropriate electron acceptor, thus

inhibiting the formation of the hydroxyl radicals that lead to the mineralisation of the target analyte. Pelaez et al. (2011) proposed that NOM might perform the same function, this, however, is unlikely as the neutral to mildly alkaline range (pH 8.0) ensures that the NOM predominantly acts as a radical species scavenger. Thirumavalavan et al. (2012) have proposed that NOM and increased turbidity can hinder the photolysis of MC-LR at the UV irradiation wavelengths employed in the current study by reducing transmittance and light absorption. This would further account for the decreased removal of MC-LR in the waste lagoon water compared with the RO filtered water. He et al. (2012) also reported that NOM had a negative impact on MC removal their study, albeit much lower concentrations of both toxin and NOM were used/present in that study which was conducted in natural water samples from a lake and a river. Autin et al. (2013) demonstrated that background organic matter not only acts as a scavenger but may also saturate the catalyst's surface in their study investigating the photocatalytic degradation of the pesticide metaldehyde by UV/H_2O_2 and UV/TiO_2. Khan et al. (2010) showed that the humic portion of NOM can also attenuate the incidental irradiation due to their light absorbing properties when investigating the photocatalysis of brevetoxins in the presence of humic acid.

High performance size exclusion chromatography (HPSEC) analysis of the protein and humic acid portion of the organic matter in waste water can demonstrate the removal of organic compounds other than the target analyte (Figure 2). Results indicate that the concentration of the humics and proteins decrease slightly in the control samples (figure 4 B and D) possibly due to photolysis in the UV only control and surface adsorption onto the TiO_2 in the dark control. More significant changes, however, were observed in the protein and humics profiles following photocatalytic

treatment (figure 4 A and C) with the general trend in reduction in apparent molecular weight for both analyte groups suggesting significant degradation has taken place, reducing larger molecules to smaller fragments. Furthermore, the overall intensity of the signal for both proteins and humics has been reduced by around a third. This confirms that photocatalysis of the NOM occurs concurrently with the photocatalysis of MC accounting for the reduced MC removal efficiency in the waste lagoon water demonstrating, as might be expected, that UV/TiO_2 oxidation is non-selective. Another indicator of this is the fact that most of the water quality parameters improve, such as UV absorbance and transmittance at 254 nm (also indicative of NOM removal), colour and turbidity (which is in part due to NOM removal and in part due to the reactor acting as a filter), and dissolved organic carbon (again indicative of NOM removal; Supporting Information, S4).

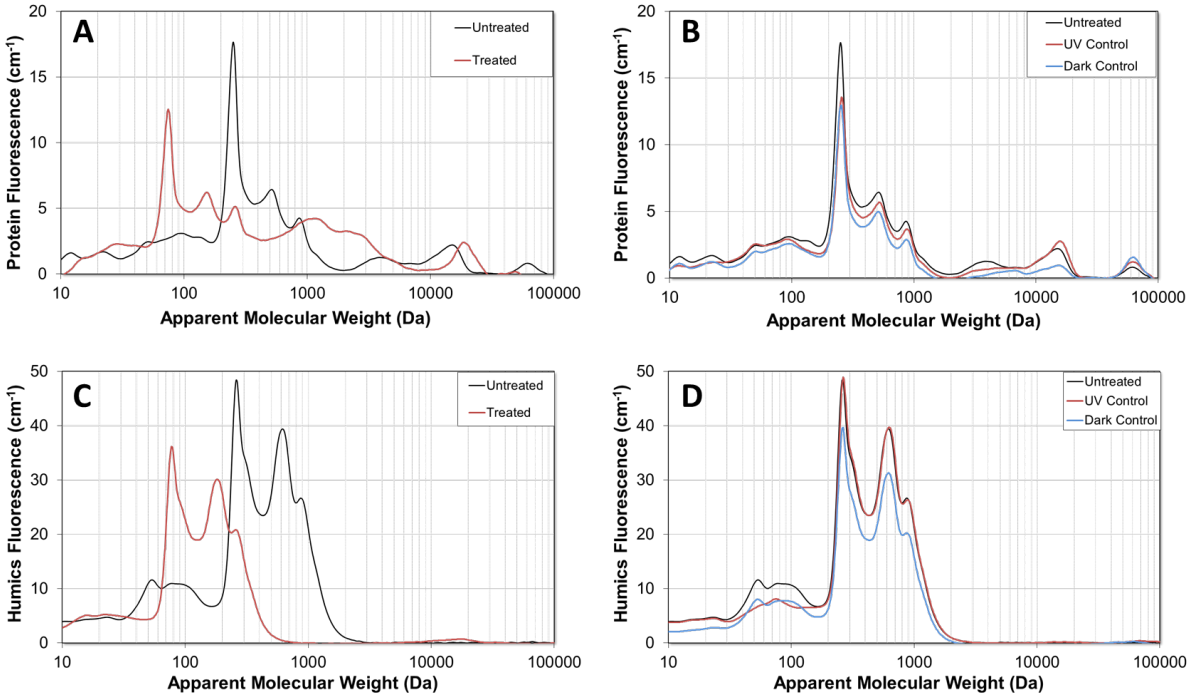


Figure 2: HPSEC determination of protein content (A and B) and humic acid content (C and D) in untreated waste lagoon samples and in UV/TiO_2 treated waste lagoon samples, and control samples (UV and dark controls).

3.2 Evaluation of MC congener destruction in waste lagoon water

Single microcystin analogues in waste lagoon water were passed through the reactor (Figure 3). The amount of dark adsorption (the initial binding of an organic pollutant to the titanium dioxide in the dark) can be an important determining factor in the removal rate of that pollutant (Feitz et al., 1999; Linda A Lawton et al., 2003). It has been observed in previous studies (Linda A Lawton et al., 2003; Pestana, 2012; Pestana et al., 2015) that the amino acid composition at the variable amino acid site impacts the dark adsorption of a given microcystin analogue.

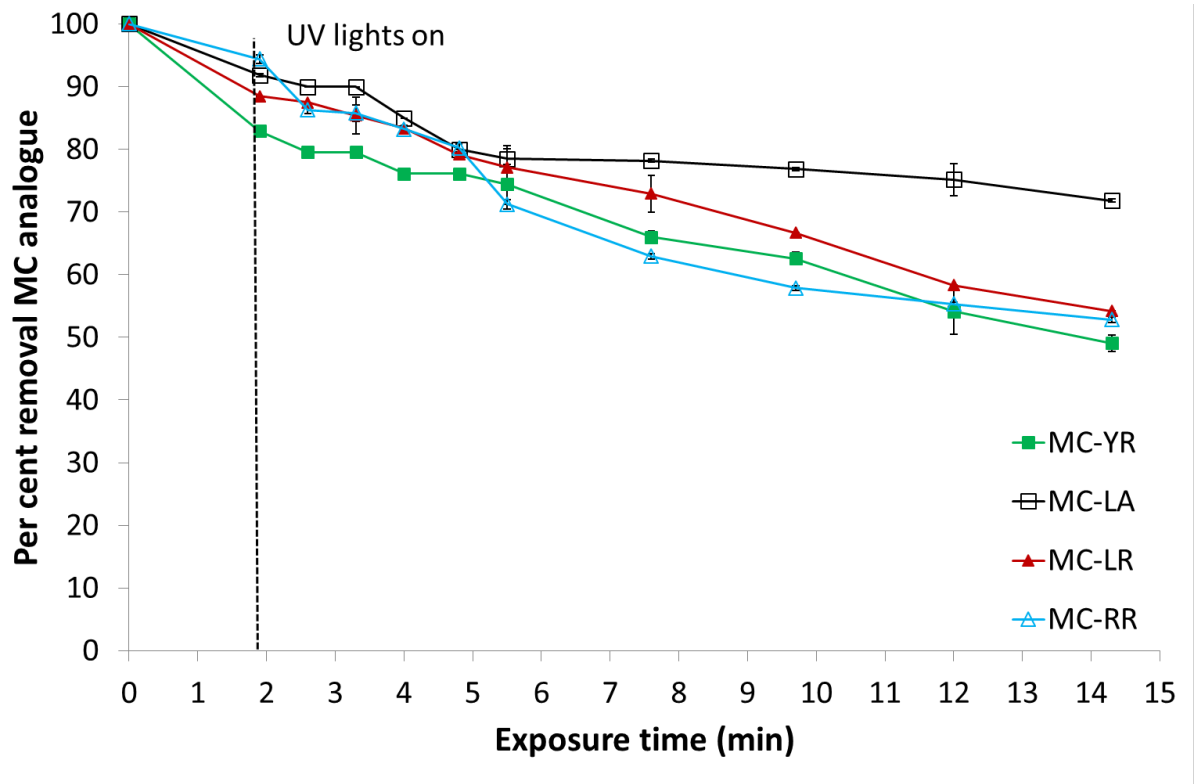


Figure 3: TiO_2/UV packed-bed flow-through reactor removal of four individual microcystin analogues (30 µg L^{-1}, respectively) in waste stabilisation lagoon water. (Error bars=1 SD, *n*=2)

In the present study, the four MC variants display slightly different dark adsorption ranging from 7% (MC-RR) to 17% (MC-YR). Direct comparison with previous studies is difficult as systems, including catalyst, light source, and physicochemical parameters vary significantly. Furthermore, the presence of NOM in the waste stabilisation lagoon water would further impact the dark adsorption of the MC analogues, due to competition for the binding sites on the catalyst, as was also observed by Autin et al. (2013). Despite the presence of NOM, and inorganic ions that can inhibit UV/TiO_2 photocatalys (Khan et al., 2010), in the water, breakdown of all four microcystin analogues can be observed. MC-YR underwent the greatest removal (51%) after 14.3 min exposure time, followed by MC-RR 48%, and MC-LR with 46% removal which all demonstrate similar efficiency. However, removal of MC-LA was much lower, with around half the efficiency (28%) showing an initial decline of around 20% in the first 5 min but removal slowing dramatically so that less than 10% is degraded in the subsequent time (9.3 min). One possible explanation for the lower oxidation of MC-LA may be due to the fact that the point of zero charge (pH_{zpc}) for TiO_2 was determined to be pH 6.25 (Hoffmann et al., 1995), which means that below this value the surface of the TiO_2 is positively charged and above this value the surface becomes negatively charged. According to Lawton and co-workers (Linda A Lawton et al., 2003), if the pH of the test solution is greater than the pH_{zpc} the oxidation of cationic species would be favoured, whereas if the pH of the test solution is lower than the pH_{zpc} the oxidation of anionic species would be favoured. This suggests that the oxidation of MC-LA is not favoured in the current study (initial pH of test solution 8.0) considering the net charge of MC-LA at pH 7.4 is -2

(Herfindal et al., 2009). While the net ionic charge of MC-YR, MC-LR, and MC-RR may have been anionic (Rivasseau et al., 1998), they are less than MC-LA (predicted charge of 0 or -1), which may explain the increased destruction of these analogues compared to MC-LA. Furthermore, it was determined by Rivasseau et al. (1998) that MC-YR is the least hydrophobic of the three MC they investigated, followed by MC-LR, and finally MC-RR at pH 7, leading to MC-LA being less likely to be adsorbed onto the catalyst surface. While surface adsorption is not essential for degradation, it has been suggested that when molecules are adsorbed the surface they are held in close proximity to the site of hydroxyl radical production (Lawton et al., 2003), hence their degradation is more efficient. Rivasseau and co-workers (1998) also found MC-YR was adsorbed (13% at pH 6.7) to natural suspended matter in river water samples slightly more so than MC-LR (11%), and MC-RR (9%). These observations are similar to those in the current study with initial adsorption: MC-YR 17 %; MC-LR 11%; MC-RR 7 %. The results in the present study agree with previous studies (Lawton et al., 2003; Pestana et al., 2015; Shephard et al., 1998), that the efficiency of removal of different microcystin analogues in a photocatalytic system will depend on the variable amino acid composition of the MC analogue (and pH of the system) due to charge, hydrophobicity influences, and the differing susceptibility of various amino acid groups to oxidation, which was demonstrated in oxidation by chlorination where a reactivity order of MC-YR>MC-RR>MC-LR>MC-LA has been found (Ho et al., 2006b).

In addition to determining the photocatalytic efficiency of the reactor with single MC analogues, a mixture of all four analogues was investigated to determine whether direct competition affected the photocatalysis of the different MC (Figure 4).

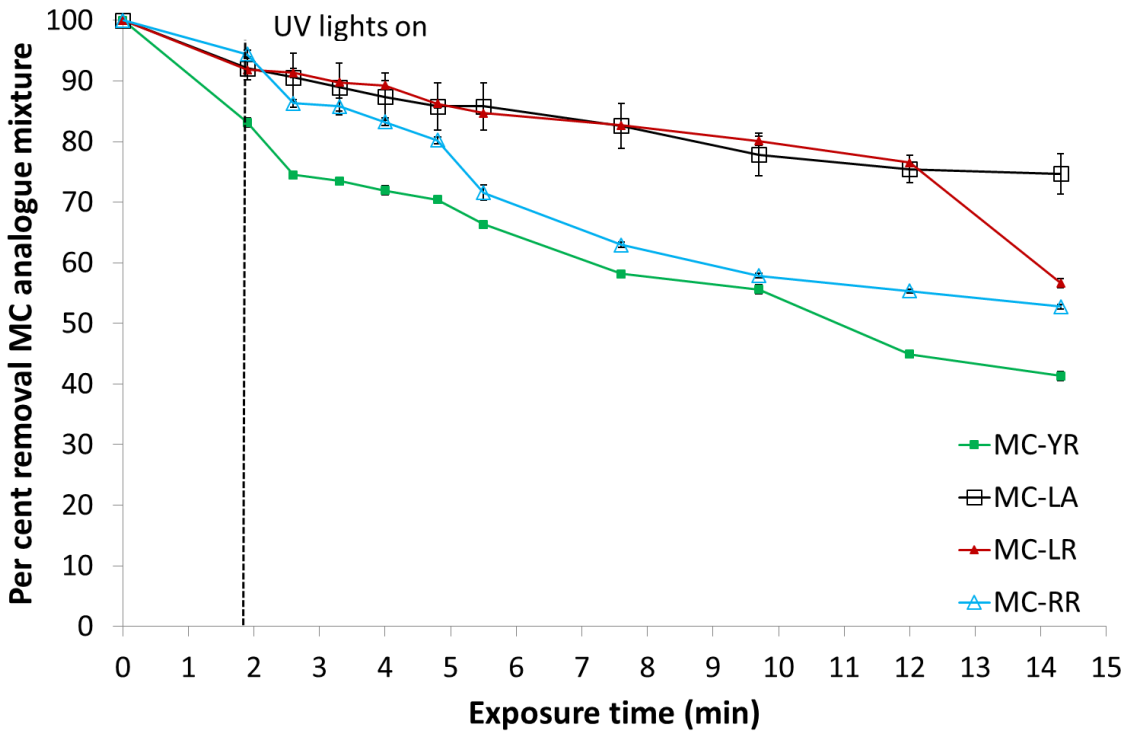


Figure. 4: TiO_2/UV packed-bed flow-through reactor removal of a mixture of four microcystin analogues (10 µg L^{-1} of each analogue) in waste stabilisation lagoon water. (Error bars=1 SD, *n*=2)

The amount of dark adsorption remains constant for three of the variants (MC-LR increases slightly from 8 to 11%) suggesting little competition between the analogues as might be expected considering the relative concentrations of the analogues compared to the other dissolved organics (DOC 23.8 mg L^{-1}; Table S3). Despite much lower background interference in their study Rimoldi et al. (2017) also reported no decreased removal of their target analytes (tetracycline, caffeine, paracetamol, atenolol) by TiO_2 photocatalysis when they were present in a mixture. The removal of MC-RR, MC-LA and MC-LR remained very similar (3% less removal for MC-LA and-LR) while the removal of MC-YR was also similar there was an increased from 51 to 59%. This confirmed that MC-YR is more readily removed than the other three analogues, regardless of whether it was treated as a single or mixed array of MC. This will be of relevance in applying this technology to naturally MC contaminated water because it has often been observed that multiple MC are

present during bloom events. Furthermore, it demonstrates the importance of not directly extrapolating predicted efficiencies from trials with different analogues.

The mechanism of the photocatalytic destruction of microcystins has been previously reported in a number of detailed studies by Liu *et al.* (2003), Song *et al.* (2007) and Antoniou *et al.* (Antoniou et al., 2008a, 2008b). Using LC-MS to identify by-products of the photocatalytic process, Liu *et al*. suggested the main processes involved in the photocatalytic decomposition process were a UV photo-isomerisation followed by hydroxyl radical attack and direct oxidation of the microcystin molecule. Song *et al*. proposed that UVC light would be required for the photo-isomerisation process and this could not be initiated by UVA light. Antoniou *et al.* [45] subsequently proposed that the isomerisation may have been induced by hydroxyl radical attack on the diene bonds of the ADDA group on the microcystin. Again using LC-MS to identify intermediates, Antoniou and co-workers [46] reported a total of eleven new intermediates that were not detected in the study by Liu *et al.* It should be noted that this study was conducted at pH 5.7 using immobilised film reactors, while Liu used a slurry reactor. The complex matrix (waste water) used in the current study prevented the elucidation of degradation (by-)products. A common concern in the degradation potentially harmful pollutants is the danger represented by degradation products displaying a similar, or, in fact, higher toxicity than the parent molecule. This is not usually the case for microcystins. As elucidated by the studies of Antoniou et al. (2008a, 2008b), the first location of hydroxyl radical attack is the ADDA moiety of the microcystin molecule. The attack in several steps changes the arrangement of this moiety (Antoniou et al., 2008a). Several studies have shown that the ADDA moiety is primarily responsible for the toxicity of microcystins (An and Carmichael, 1994; Dawson, 1998; Luukkainen et al., 1994; Trogen et al., 1996), therefore it can be

concluded that the degradation intermediated of the photocatalytic removal of microcystins does not lead to the creation of more toxic compounds, but rather a detoxification of the solution.

3.3 Photocatalysis of *Microcystis aeruginosa* cells

Evidence from the literature suggests that titanium dioxide is capable of inactivating cyanobacteria (Hong et al., 2005; Kim and Lee, 2005). According to Malato et al. (2009) the susceptibility of microorganisms to photo-inactivation is (from least to most susceptible): protozoa, bacterial spores, mycobacteria, viruses, fungi, bacteria. Furthermore, there is a ranking of susceptibility within the group of bacteria with Gram-positive bacteria being less susceptible than Gram-negative bacteria due to differences in the structural complexity of the cell wall (Lydakis-Simantiris et al., 2010). According to the aforementioned ranking the common MC-producing cyanobacteria *M. aeruginosa* which has a Gram negative cell wall should show susceptibility to photocatalytic inactivation. Treatment in the packed-bed flow-through reactor found that the photocatalytic process had no effect on cell numbers (Supporting Information, S5). The number of cells remains stable in the treatment as well as the two controls (UV control and dark control). It is likely that reactor design, while effective for dissolved MC, is not appropriate for the treatment of cyanobacterial cells. When successful removal of microorganisms is reported in the literature they typically report the use of a static system, nano-particulate or thin film catalysts, and/or long contact times (Baram et al., 2011; Benabbou et al., 2011; Hong et al., 2005; Kim and Lee, 2005; Prasad et al., 2009; van Grieken et al., 2009a, 2009b). The flow-through system used in this study provided only limited treatment exposure (14.3 mins), which may not be sufficient considering other studies (Hong et

al., 2005; Kim and Lee, 2005) where test solutions were exposed between 60 mins and four days. The study did, however, show that extracellular MC (MC-LR, -YR, -RR) released from the *M. aeruginosa* were photocatalysed (figure 5).

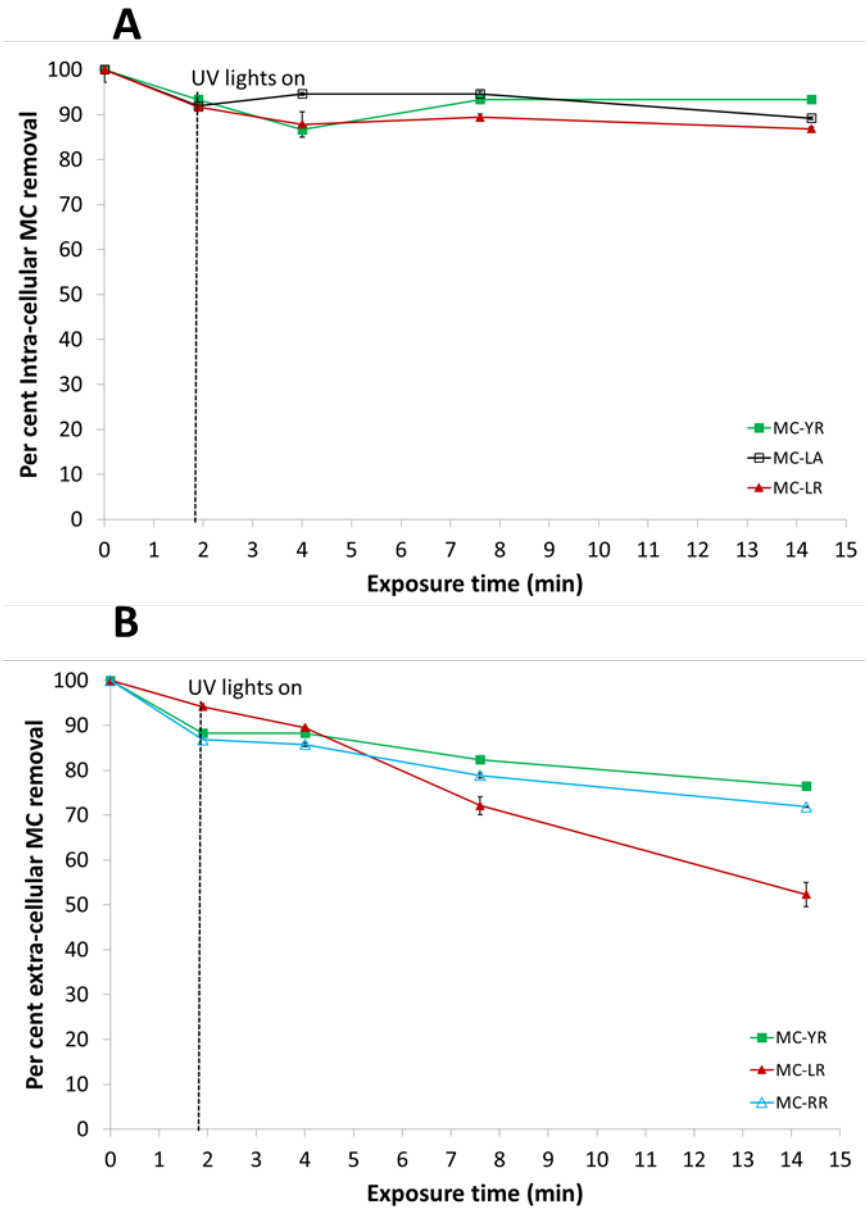


Figure 5: TiO_2/UV packed-bed flow-through reactor removal of intra- (A) and extra-cellular (B) MC during the photocatalytic treatment of *M. aeruginosa* in waste stabilisation lagoon water. (Error bars=1 SD, *n*=2)

High concentrations of MC-RR (68 µg L^{-1}) were found to be present in the culture and a reduction in MC-RR concentration of 28% was observed. Lower concentrations of MC-LR and MC-YR were also detected (8.6 and 3.4 µg L^{-1},

respectively) which also degraded (48 and 24%, respectively). These findings demonstrate that while whole cyanobacterial cells were unaffected by photocatalysis, the levels of dissolved MC analogues were reduced. A further indicator that the cells were not disrupted cell is that intra-cellular MC levels do not change during the photocatalysis.

3.5 Conclusion

The prevalence of microcystins in waste water can have serious implications for public health and safety depending on waste water effluent utilisation. Irrigation of food crops or of recreational areas can lead to human and animal intoxication, release into streams/rivers carries similar risks. This investigation has demonstrated the practical application of TiO_2 photocatalysis for waste stabilisation lagoon effluent treatment. The current reactor configuration readily lends itself to a larger scale application: The packed-bed nature of the design removes the challenge of catalyst separation post treatment and the continuous flow ensures that effluent can constantly be treated without the need to revert to a batch application. One area the current reactor design could be improved on is the UV irradiation source, however, it was demonstrated in a previous study (Pestana, 2012) that UV light emitting diodes could easily replace the need for high powered and energy inefficient UV lamps. The presence of NOM in waste water effluent challenges the removal of MC, but as has been demonstrated does not negate the removal by photocatalysis. Using a TiO_2 packed-bed flow-through reactor full-scale application could provide a low maintenance, low cost treatment for the elimination of cyanotoxins (and other trace toxic pollutants) in low quality water in a format which could be deployed in diverse environments across the globe.

Supporting Information

Additional information about the rationale behind catalyst selection (section S1), a detailed description of the reactor design (section S2), light and dark controls for the removal of MC-LR from waste stabilisation lagoon water (section S3), a detailed analysis of water quality parameters in untreated, UV/TiO_2 treated and control samples (section S4), and cell viability data for the photocatalysis of *M. aeruginosa* (section S5).

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Supporting Information for: Removal of microcystins from a waste stabilisation lagoon: Evaluation of a packed-bed continuous flow TiO_2 reactor

Carlos J. Pestana[a,b]*, Peter Hobson[a], Peter K. J. Robertson[c], Linda A. Lawton[b], Gayle Newcombe[a]

[a] Australian Water Quality Centre, South Australian Water Corporation, 250 Victoria Square, Adelaide, SA 5000, Australia

[b] School of Pharmacy and Life Sciences, Robert Gordon University, Sir Ian Wood Building, Garthdee Road, AB10 7GJ, Aberdeen, UK

[c] School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, 39 Stranmillis Road, BT9 5 AG, Belfast, UK

* To whom correspondence should be addressed: c.pestana@rgu.ac.uk

The Supporting Information contains 3 tables and 4 figures, totalling 14 pages.

Table of Contents:

S1 Rationale of TiO_2 catalyst selection

Hombikat K01/C pelletised TiO_2 catalyst was chosen for this reactor design, because it has previously been shown to successfully degrade microcystins (Liu et al., 2009). Some of its features include, large robust pellets (table S1) which retain integrity when tightly packed in the reactor, hence no requirement for powdered catalyst removal post-treatment (Pestana et al., 2014; Robertson et al., 2011) and lends itself to a scalable reactor design as good flow and contact are achieved (Pestana et al., 2014). The surface area of 42 $m^2\,g^{-1}$ is similar to the well-established nano-particulate Degussa P25, which has a surface area of 50 $m^2\,g^{-1}$ (figure S1). This is surprising, although it was thought that the larger particles would naturally exhibit much lower surface area, it would appear that they remain porous hence exhibiting a higher than expected surface area (Liu et al., 2009).

Table S1: Characteristics of pelletised Hombikat K01/C and nano-particulate Degussa P25 TiO_2 catalysts[1].

Hombikat K01/C TiO_2 pellets	
Length	8-15 mm
Diameter	~ 5 mm
Surface Area	42 $m^2\,g^{-1}$
TiO_2	100%
crystalline composition	anatase
Degussa P25 TiO_2 nano-particulates	
Particle Size	20 nm
Surface area	50 $m^2\,g^{-1}$
TiO_2 crystaline composition	75% anatase, 25% rutile

Figure S1: Photograph and SEM picture (Pestana, 2012) of Hombikat K01/C TiO2 pellets (left) and Degussa P25 nano-particulates (right).

S2 Photocatalytic reactor design

The reactor design evolved through an iterative approach which included catalyst suitability (S1), material selection and illumination (Liu et al., 2009; Pestana, 2012; Pestana et al., 2014; Robertson et al., 2011). A bench-top design was selected and informed from previous studies (Pestana et al., 2014) to facilitate detailed investigation of the removal of MC variants. Furthermore, larger volumes would have been prohibitive due to the cost of purified MC (1 mg MC-LR ~ US$ 500; 1 mg MC-RR ~ US$ 1200; 1 mg MC-LA ~US$ 2850; 1 mg MC-LY ~ US$ 5100; Enzo Life Sciences, UK). The reactor was designed with a recycling system (figure S2) to allow a compact design and evaluation of increasing contact/irradiation time. It was constructed using Plexiglass (Evonik, Germany) with >98% UV permeability and consisted of three parallel channels (channel dimensions 300 x 64 x 18 mm), connected at alternating ends by a 10 mm gap in the dividing wall allowing water to pass in a serpentine manner through the reactor. Total reactor dimensions were 300 x 250 mm, hence total path length was 900 mm.

The sides of the reactor were sealed with polycarbonate end pieces. The end pieces had a threaded hole cut into them that allowed the attachment of 7 mm diameter stainless steel nozzles (RS Components, United Kingdom) to facilitate the attachment of silicone tubing to the reactor. The reactor was packed with pre-washed Hombikat K01/C titanium dioxide pellets (692 g). The fluid volume of the filled reactor was 300 mL at room temperature. Waste stabilisation pond water flow through the reactor was achieved by peristaltic pump with 6 mm silicone tubing (Altasil, USA; flow rate 0.5 L min^{-1}) from a 3 L reservoir. The UV lamp (Dermfix 3000 UV lamp, consisting of two UV narrowband PL-L 36 W tubes with a 12.4 W combined UV-B output, according to the manufacturer, Dermfix, Germany) was placed 200 mm from the surface of the reactor.

In a typical experiment, after allowing the system to reach equilibrium for 18 min, the system was irradiated for 90 min. The actual exposure times, corrected to allow for the length of time water resides in the reactor and for a diminishing experimental volume due to sampling (equation S1) are given in table S2.

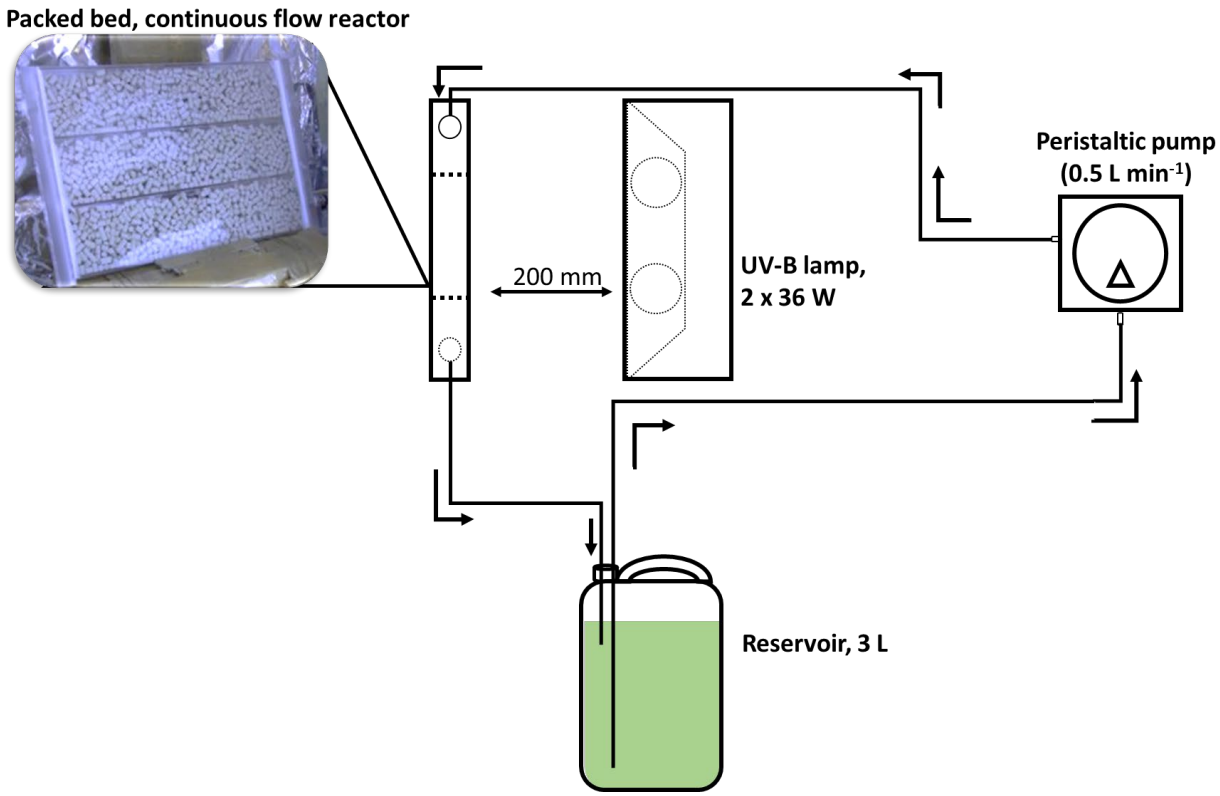

Figure S2: Design of packed-bed flow-through photocatalytic reactor for the treatment of microcystins and cyanobacterial cells in waste stabilisation lagoon water. Arrows indicate direction of flow.

Equation S1: Equation used to determine actual exposure time.

$$exposure\ time\ at\ irradiance\ time\ (t_i) = \left(\frac{t_i/t_e}{V_E/V_R}\right) \times\ t_e$$

Where,

t_i is the irradiance time of the system,

t_e is the exposure time of one reactor volume of water, in this case it was constant at 0.6 min,

V_E is the experimental volume at selected sampling point,

V_R is the reactor volume, in this case 0.3 L.

Table S2: Sampling times during treatment and actual exposure times based on flow and reactor volume.

Sampling time (min)	Actual exposure time (min)
0	0
18	1.9
UV lights on	
24	2.6
30	3.3
36	4.0
42	4.8
48	5.5
63	7.6
78	9.7
93	12.0
108	14.3

S3 UV and dark controls of TiO_2 photocatalysis of MC-LR

Two controls, one in the absence of light and one in the absence of the catalyst, were performed to determine loss of MC-LR that was not a result of photocatalysis (figure S3). These full cycle controls were only performed with MC-LR, the initial concentration was 10 µg L^{-1}, in waste stabilisation lagoon water. The initial removal of approximately 12% observed for the dark control (absence of light) is typically referred to as dark adsorption onto the catalyst (Liu et al., 2009) with continued slight reduction due to system loss or further dark adsorption. Similarly, 5% removal of microcystin-LR in the UV control (in the absence of catalyst) is most likely also due to system loss.

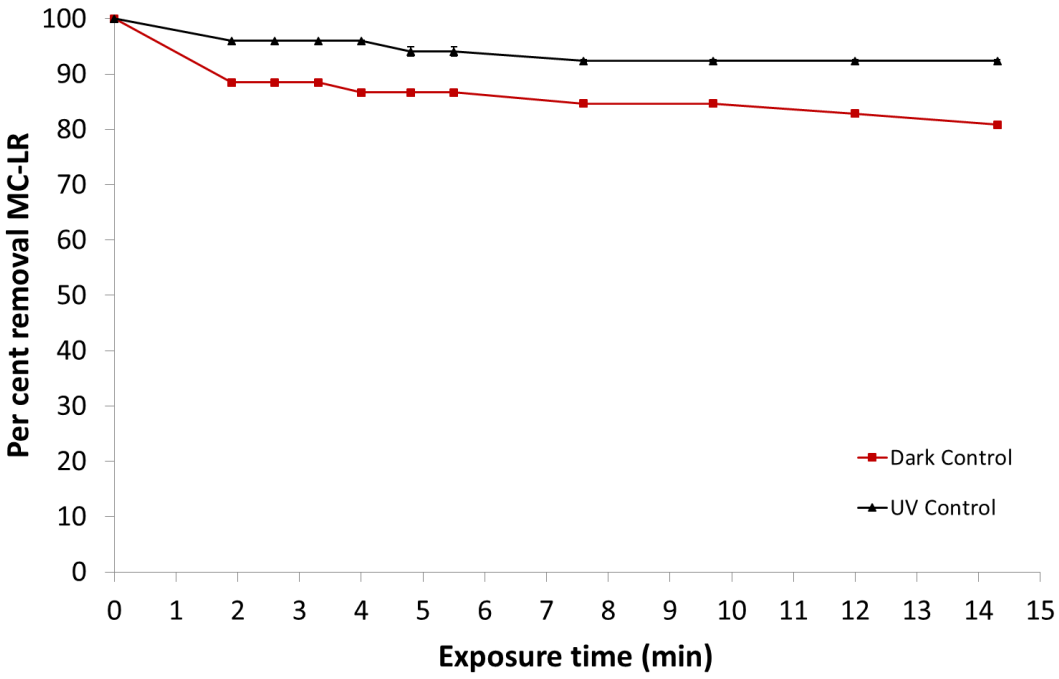

Figure S3: HPLC-PDA determination of UV and dark controls of MC-LR (10 µg L^{-1}) in TiO_2 packed-bed flow-through reactor in waste lagoon water. UV control performed in the absence of catalyst; dark control performed without illumination. Error=1SD, *n*=2.

S4 Water Quality Analysis before and after UV/TiO_2, UV, and TiO_2 treatments

Water quality analysis of the untreated, UV/TiO_2, UV, and TiO_2 treated samples was performed (table S2). Samples were filtered (0.45 µm) for UV_{254} analysis and DOC analysis and filtered (0.22 µm) for colour analysis. The UV_{254} absorbance and transmission was measured through a 1 cm quartz cell and colour (true and apparent) was measured through a 5 cm quartz cell using an Evolution 60 Spectrometer (Thermo Scientific, USA). The DOC was analysed by a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA). Turbidity was determined using unfiltered samples with a 2160AN Turbidimeter (Hach, USA). Conductivity of the samples was measured using a LF340 conductivity-

meter and pH was measured using a pH315i pH-meter (both WTW, Germany).

Table S3: Impact of photocatalysis in a TiO_2 packed-bed continuous-flow reactor on the water quality of water from a waste stabilisation lagoon and the effect of the addition of hydrogen peroxide (50 mg L^{-1}) as a photocatalytic enhancer.

Parameter	Untreated	UV/TiO_2	UV	TiO_2
UV_{254} Absorbance	0.435	0.323	0.419	0.428
UV_{254} Transmittance	37%	48%	38%	37%
Colour – true	45 HU	34 HU	44 HU	45 HU
Colour – apparent	302 HU	206 HU	299 HU	300 HU
Turbidity	34.1 NTU	12.1 NTU	32.2 NTU	33.5 NTU
pH	8.01	7.5	7.8	7.8
Conductivity	1173 µS cm^{-1}	976 µS cm^{-1}	1135 µS cm^{-1}	1154 µS cm^{-1}
DOC	23.8 mg L^{-1}	20.3 mg L^{-1}	23.7 mg	22.9 mg

UV_{254} absorbance is a measure of the organic content of a water sample. Light at 254 nm is readily absorbed by organic matter and is a rapid measure to determine organic content in water (APHA, 2012). As indicated by other measurements, the treated samples display a reduced absorbance value compared to the untreated sample, indicating that organic matter is removed alongside the microcystins. UV_{254} transmittance is a complementing measure to UV_{254} absorbance, it describes the percentage of 254 nm light passing through a sample, i.e. it measures the light not absorbed (WHO, 2017). An increase in the light transmittance, as observed for both treated samples, indicates the

removal of organic compounds adsorbing at 254 nm. $Colour_{456}$ describes the colour of a water sample determined at 456 nm and compared to a platinum/cobalt standard. Results are expressed as Hazen Units (HU) which equates to a μg L^{-1} concentration of a solution of potassium hexachloro-platinate(IV) and cobalt(II)chloride (APHA, 2012). For true color the sample is filtered (0.22 μm) to remove suspended particles, for apparent it is not. There is no World Health Organization (WHO) guideline for color (true or apparent), but based on aesthetics, a value below 15 HU is recommended (WHO, 2017). In the current investigation the true color is reduced by the photocatalytic treatment, but does not reach the recommended values by the WHO, bearing in mind that the recommendations are for potable water and not for waste water.

Turbidity is a measure of the haziness of water due to suspended or colloidal particles (organic or inorganic). Turbidity is commonly measured in nephelometric turbidity units (NTU); the WHO recommends values of no more than 5 NTU and ideally below 1 NTU (WHO, 2017). During the treatment the turbidity is markedly decreased further indicating the efficacy of the treatment.

Conductivity is a measure of inorganic ions such as nitrate, sulphate, magnesium and iron which can interact with the catalyst surface inactivating it (Umar and Aziz, 2013). This could account for a degree of the reduced efficacy observed although this would require further clarification.

S5 Photocatalytic removal of *Microcystis aeruginosa*

Microcystis aeruginosa 338 Kutz. emend Elenkin (Australian Water Quality Centre) was cultured in ASM-1 medium at 20 °C under a 12h/12h light/dark cycle at an intensity of 70 µmol photons $m^{-2}s^{-1}$. This strain produces MC-LR, -YR, -RR, and -LA.

Cells of *M. aeruginosa* (3.0 x 10^5 cells mL^{-1}) were exposed to the TiO_2 packed-bed continuous-flow photocatalytic reactor in the same experimental set-up as the dissolved MC investigations. Cell numbers, viability and both intra and extra-cellular MC was determined. In the current reactor no removal of *M. aeruginosa* was observed (figure S4), which could be due to insufficient contact time or the pelletised nature of the TiO_2. In a previous study nano-particulate powdered TiO_2 was effective in removing *Escherichia coli*, however, the reactor configuration was very different from the current design and UV irradiation was conducted 10 times longer (105 min) (Robertson et al., 2015). Similar differences exist with another study that demonstrated the successful removal of *M. aeruginosa* and a green alga in a lake utilising TiO_2 coated glass spheres (Kim and Lee, 2005). While no removal of *M. aeruginosa* could be demonstrated, extra-cellular MC was reduced (figure 5, main article).

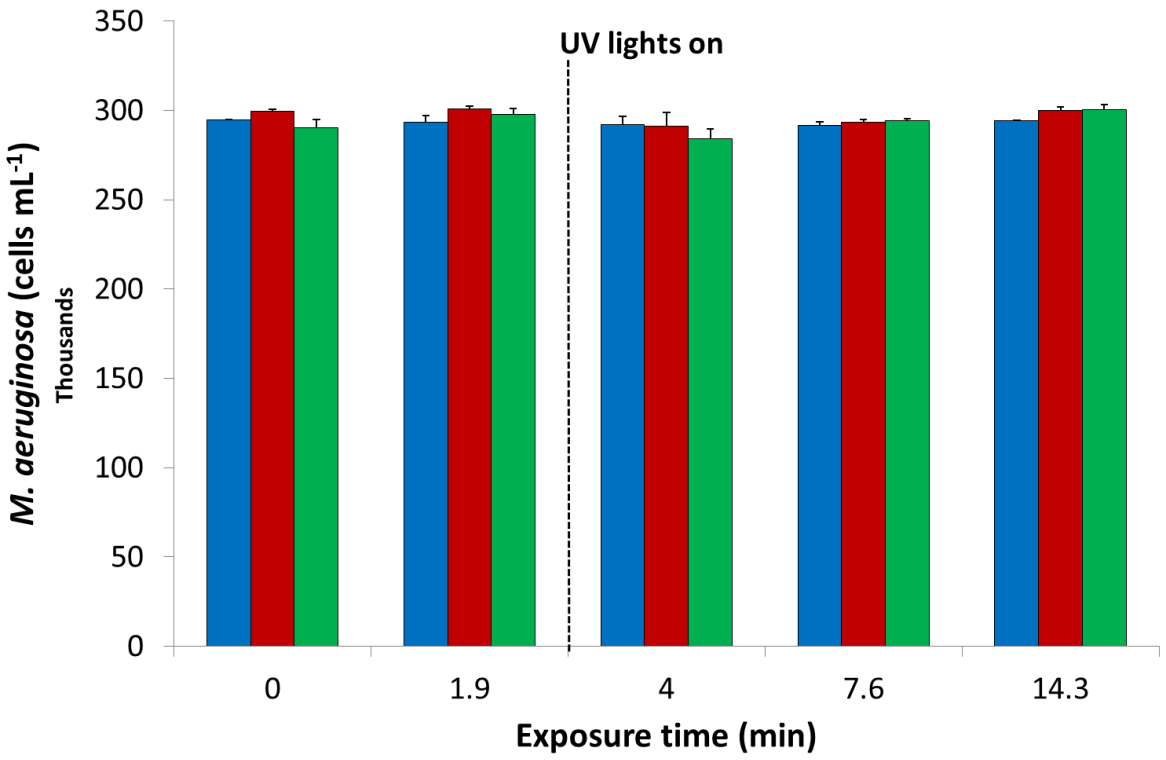

Figure S4: Viable cells of *M. aeruginosa* (determined by SYTOX Green and FDA fluorescent stains) during TiO_2 photocatalysis in packed-bed continuous-flow reactor (blue), UV control (no catalyst; red) and dark control (no UV light; green).

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