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Cyanobacterial and microcystins dynamics following the application of hydrogen peroxide to waste stabilisation ponds

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Abstract. Cyanobacteria and cyanotoxins are a risk to human and ecological health, and a hindrance to biological wastewater treatment. This study investigated the use of hydrogen peroxide (H_2O_2) for the removal of cyanobacteria and cyanotoxins from within waste stabilization ponds (WSPs). The daily dynamics of cyanobacteria and microcystins (commonly occurring cyanotoxins) were examined following the addition of H_2O_2 to wastewater within both the laboratory and at the full scale within a maturation WSP, the final pond in a wastewater treatment plant. Hydrogen peroxide treatment at concentrations ≥ 0.1 mg H_2O_2 μg^{-1} total phytoplankton chlorophyll *a* led to the lysis of cyanobacteria, in turn releasing intracellular microcystins to the dissolved state. In the full-scale trial, dissolved microcystins were then degraded to negligible concentrations by H_2O_2 and environmental processes within five days. A shift in the phytoplankton assemblage towards beneficial Chlorophyta species was also observed within days of H_2O_2 addition. However, within weeks, the Chlorophyta population was significantly reduced by the re-establishment of toxic cyanobacterial species. This re-establishment was likely due to the inflow of cyanobacteria from ponds earlier in the treatment train, suggesting that whilst H_2O_2 may be a suitable short-term management technique, it must be coupled with control over inflows if it is to improve WSP performance in the longer term.

1 Introduction

Cyanobacteria are a common occurrence in waste stabilisation ponds (WSPs), a suspended growth biological treatment technology used in outdoor municipal wastewater treatment plants (WWTPs) (Furtado et al., 2009; Ho et al., 2010; Martins et al., 2011; Vasconcelos and Pereira, 2001). Several genera produce toxins, including microcystins, which pose a significant health risk to humans and animals. Both cyanobacteria and their toxins (cyanotoxins) are thus a substantial threat to ecological systems (Christoffersen, 1996; de Figueiredo et al., 2004).

The presence of cyanotoxins in drinking and recreational waters is often of greater concern to water management authorities than their presence in WSPs. However, as well as their inherent risk to human and ecological health, cyanobacteria are a hindrance to the wastewater treatment processes (Martins et al., 2011). Cyanobacteria and cyanotoxins inhibit the biological, physical, and chemical treatment of wastewater. Hence, the presence of cyanobacterial blooms increases the possibility that inadequately treated effluent will be discharged, possibly containing harmful cyanotoxins.

Previous investigations into the removal of cyanobacteria and cyanotoxins have generally been conducted on single species cultures and purified toxins under laboratory conditions. Limited knowledge is available regarding their removal from WSPs, even though treated wastewater is often used for irrigation or returned to the environmental flow (Toze, 2006). Although it is common practice to remove cyanobacteria using copper sulfate, chlorine, or coagulants

and flocculants (Hrudey et al., 1999), the dynamics of the removal of cyanobacteria and cyanotoxins from wastewater have not been thoroughly investigated. Additionally, the cyanobacterial removal methods currently practiced may negatively impact the environment, and may not be effective at removing cyanotoxins (Hrudey et al., 1999; Kenefick et al., 1993).

Hydrogen peroxide (H_2O_2) has shown promise in reducing cyanobacteria and cyanotoxin concentrations, with increased success when coupled with other chemicals or radiation (Cornish et al., 2000; Drábková et al., 2007; Matthijs et al., 2012). Hydrogen peroxide does not accumulate in the environment, as it is decomposed rapidly to water and oxygen gas via biological, chemical and photochemical mechanisms (Cooper et al., 1994; Drábková et al., 2007). The chemical inhibits photosynthetic activity in cyanobacteria by impairing electron transfer and oxygen evolution, which can lead to the inactivation of photosystem II, and eventually cellular death (Samuilov et al., 2004). Because cyanobacteria are prokaryotic, this probably occurs more rapidly than when H_2O_2 is applied to eukaryotic cells, such as chlorophytes, whose photosynthetic apparatus are contained within discrete organelles. This suggests that H_2O_2 may be selective for cyanobacteria in mixed phytoplankton assemblages (Barrington and Ghadouani, 2008; Barroin and Feuillade, 1986). Hydrogen peroxide also forms hydroxyl and hydroperoxyl radicals which destroy the toxicity of cyanotoxins, such as microcystins, by inducing oxidative cleavage (Antoniou et al., 2008).

Following the addition of H_2O_2, the dynamics of cyanobacteria and cyanotoxins must be investigated before it can be recommended for use in cyanobacterial control. Most previous studies into the removal of cyanobacteria and cyanotoxins using H_2O_2 have not assessed the potential consequences of environmental and WSP conditions, although a recent study by Matthijs et al. (2012) has investigated the application of H_2O_2 to a lake. Many biological, chemical and physical variables may moderate the effective reduction of ecological assemblages of cyanobacteria and cyanotoxins by H_2O_2 (Barrington et al., 2011; Harada and Tsuji, 1998; Matthijs et al., 2012).

It has been suggested that when released from cyanobacterial cells, dissolved cyanotoxins may be degraded by environmental mechanisms within days to weeks (Harada and Tsuji, 1998). Hydrogen peroxide promotes cell lysis, and if cyanotoxins are not degraded by the chemical itself, their degradation by natural mechanisms is of significance. It has been demonstrated that the bacteria found in activated sludge effluent (produced during wastewater treatment) are able to degrade cyanotoxins, particularly microcystin species (Ho et al., 2010; Lam et al., 1995). In particular, Ho et al. (2010) compared the degradation rates of dissolved microcystins in both fresh activated sludge treated effluent and axenic tertiary-activated sludge treated effluent within the laboratory. Ho et al. (2010) determined that indigenous bacteria in WSPs are capable of degrading microcystins within days. This study also found that there was little reduction of microcystins in sterilised water, indicating that under laboratory conditions, biodegradation processes are more likely to remove microcystins than physical processes (Ho et al., 2010).

The work presented here investigates the effects of H_2O_2 addition on cyanobacteria and cyanotoxins, particularly microsystins, both within the laboratory and at a full-scale WSP. More specifically, the objectives were to (1) determine the dynamics of intracellular and dissolved microcystins following H_2O_2 treatment, (2) investigate the coupling between cyanobacterial lysis and microcystins release following the addition of H_2O_2 to a large scale cyanobacterial bloom under environmental conditions, and (3) investigate the longer term ($\sim$ one month) effects of H_2O_2 treatment on the WSP assemblage.

2 Methods

2.1 Study site

Laboratory trials were performed to determine the dynamics of cyanobacteria and microcystins removal by various H_2O_2 concentrations at the microcosm scale, followed by H_2O_2 application to a full-scale WSP. Samples from the Water Corporation's Merredin WWTP, a suspended growth biological treatment plant in the Wheatbelt Region of Western Australia, were selected for the laboratory trial. During the summer months, the phytoplankton assemblage in the Merredin WWTP is predominantly cyanobacteria of the genera *Microcystis* and *Planktothrix*, both known to produce microcystins. The full-scale trial was performed on a large cyanobacterial bloom in the 8091 m^3 maturation WSP. The maturation WSP is the last pond in the WWTP, and such ponds are designed to be aerobic throughout (Tchobanoglous et al., 2003).

2.2 Experimental design – laboratory trials

Two trials were designed to investigate the effects of H_2O_2 on cyanobacteria and cyanotoxins within a controlled laboratory environment. In both trials, four 20 L glass aquariums were utilised so that microcystins could not be adsorbed to the microcosms (Ikawa et al., 1999), and covered in black polyethylene so that light could only enter via the free-water surface. The temperature of both trials was kept constant at 25 °C. The microcosms were subjected to low levels of light provided by two GE Polylux XLR860 Daylight 3250 Lm fluorescent tubes. The samples were illuminated in a 13 h : 11 h light : dark cycle, reminiscent of the daylight cycle at the Merredin WWTP at the time of sample collection.

Previous studies into the use of H_2O_2 for cyanobacterial removal have defined concentrations of H_2O_2 as mass per unit volume of water. This definition does not consider that the concentration of H_2O_2 which will be effective in removing cyanobacteria and microcystins is determinant upon the

amount of H_2O_2 consumed in competing reactions within the receiving water reservoir. Hydrogen peroxide will react with phytoplankton cells other than cyanobacteria as well as organic and inorganic matter other than cyanotoxins.

Wastewater contains many biological and chemical constituents and, to allow for the use of H_2O_2 in various WSPs, a H_2O_2 concentration dependent upon a minimal number of such variables was required. It was established that within this study the most appropriate definition of H_2O_2 concentration could be determined by considering the mass of H_2O_2 per unit of phytoplankton chlorophyll *a* (chl *a*), assuming that this also related to the magnitude of organic and inorganic matter within the WSP. Concentrations were calculated according to the measurement of total phytoplankton pigment concentration (defined as chl *a*) in the water sample, determined by spectrofluorometry. This concentration definition is specific only to municipal wastewater concentrations, and would likely need to be adjusted if H_2O_2 were to be used within industrial wastewater, drinking water, or natural reservoirs. Although this concentration definition does make assumptions regarding the reactions between H_2O_2 and non-target organisms and compounds, for such work it is a more accurate dose definition than using a mass of H_2O_2 per unit volume of water. This definition goes some way to considering competing reactions which will consume H_2O_2, without requiring extensive knowledge of the thousands of such reactions that occur under environmental conditions.

The first laboratory trial consisted of two control microcosms and two treated microcosms (0.1 mg H_2O_2 μg^{-1} chl *a*, the concentration of H_2O_2 determined a priori to be appropriate for addition under field conditions (Barrington et al., 2011). As the cyanobacterial concentration present was exceptionally high ($\sim 25\,000\,\mu g$ chl $a\,L^{-1}$), the required H_2O_2 concentration was $4000\,mg\,L^{-1}$, which may be impractical for application to entire water bodies for economic reasons. However, such a dose may be applicable where surface scums, and not entire water bodies, require treatment. At the time of H_2O_2 addition, the pH of the microcosms was ~ 7.8. A second laboratory trial was performed using lower concentration cyanobacterial assemblages ($\sim 600\,\mu g$ chl $a\,L^{-1}$). A control and three microcosms, each with a different concentration of H_2O_2 (0.02 mg H_2O_2 μg^{-1} chl *a*, 0.2 mg H_2O_2 μg^{-1} chl *a* and 2 mg H_2O_2 μg^{-1} chl *a*) were prepared. Although this did not allow for replication, it was felt that trialing a variety of H_2O_2 concentrations would be beneficial to determining a suitable concentration to add in the full-scale trial. At the time of H_2O_2 addition, the pH of the microcosms was ~ 8.6. Within both laboratory trials, samples were collected for chl *a* and microcystins analysis twice on the day of H_2O_2 treatment, and once daily for four days following treatment.

2.3 Experimental design – full-scale field trial

To investigate the dynamics of cyanobacteria and microcystins following H_2O_2 treatment under field conditions, a trial was performed in the maturation WSP at Merredin WWTP. As no Southwest Australian WWTPs consisting of parallel ponds suffered cyanobacterial blooms during the fieldwork period, there were no ponds available to act as controls or replicates. However, the bloom in the maturation WSP was predominantly cyanobacteria, and contained microcystins, and was thus chosen as an appropriate full-scale field trial site.

To achieve a H_2O_2 concentration of 0.1 mg H_2O_2 μg^{-1} chl *a*, 1280 L of 50 % H_2O_2 was added to the WSP. The H_2O_2 was diluted with wastewater prior to addition, and was added via a surface jet to various points around the pond to ensure the maximum application across the surface. In previous work we determined that horizontal mixing by jet addition and wind-driven mixing was sufficient for spreading H_2O_2 throughout WSPs (Barrington et al., 2011). More H_2O_2 was added to the upwind side of the pond to ensure the maximum wind-driven spread of the chemical.

Following the addition of H_2O_2 to the Merredin maturation WSP, water was released gradually from the pond, and flow rates measured daily. It was determined from flow rate and pond volume measurements that retention time within the pond during the sampling period was approximately four weeks. Water samples representative of the top 50 cm of the water column were collected throughout this period, four times per week at the outlet of the WSP using a Van Dorn sampler. Weekly grab-samples were also taken at both the inlet and outlet of the WSP for microscopic phytoplankton cell counts.

2.4 Phytoplankton biomass

Phytoplankton biomass was primarily quantified through spectrofluorescence by use of the bbe Moldaenke FluoroProbe. During each sampling period, in each mesocosm and in the full-scale trial, ten measurements of cyanobacterial biomass were taken using the spectrofluorometer and their average calculated. The standard deviation of these ten measurements was always $< 10\,\%$ of the average value.

Spectrofluorescence measures the fluorescence of photosynthetic pigments using light emitting diodes at various wavelengths (Beutler et al., 2002; Ghadouani and Smith, 2005), and discriminates between different phytoplankton groups by measuring their fluorescence spectra. The FluoroProbe processing software converts the fluorescence of the pigments chl *a*, chlorophyll *c*, phycocyanobilin, phycoerythrobilin, fucoxanthin, and peridinin into units of equivalent chl *a* (Beutler et al., 2002; Gregor and Marsalek, 2004). This method allows for the determination of fluorescence contributed by the phytoplankton groups Chlorophyceae,

Cyanophyceae, Dinophyceae and Bacillariophyceae, and Cryptophyceae.

The FluoroProbe has been used as a measure of phytoplankton photosynthetic activity in previous studies (e.g. Beutler et al., 2002; Gregor and Marsalek, 2004), and was determined to be an appropriate proxy measurement for phytoplankton biomass within this work. The measurement of in vivo fluorescence allows the detection of changes to photosystem II, which indicates when cells are experiencing photoinhibition or photochemical inhibition (Samuilov et al., 1999, 2004). Although phytoplankton cells have repair mechanisms for damage to photosystem II, where the damage is severe, cells will likely die. The resulting absence of fluorescence indicates a reduction in phytoplankton biomass (Masojídek et al., 2011).

Additionally, weekly microscopic phytoplankton cell counts were performed by preserving samples in Lugol's iodine and adding to a Sedgewick Rafter Chamber. Samples were enumerated to the genus level by counting 25 to 30 fields within the chamber, according to the methods detailed in Hötzel and Croome (1999).

2.5 Microcystins concentration determination

In each measurement period one sample was collected for dissolved cyanotoxin analysis. This sample was filtered through 47 mm GF/C papers immediately following collection. Filter papers containing the intracellular microcystins fraction were frozen in triplicate at −20 °C prior to toxin extraction. Methanol was added to the filtrate (which contained the dissolved microcystins fraction) at 1 % (v/v).

Intracellular microcystins were extracted in triplicate for each sampling interval, using an adaptation of the methods described by Lawton et al. (1994). Filters were freeze thawed (thaw cycle at 20 °C, freeze cycle at −20 °C) three times to lyse cyanobacterial cells prior to methanol extraction. Thawed filters were submerged in 5 mL of 75 % methanol in nanopure water (v/v), sonicated on ice for 25 min, and shaken on a rotary plate for 25 min. The extracts were separated from filter papers and centrifuged at 3750 rpm for 10 min at room temperature. The supernatant was transferred into a conical flask, and each filter paper further extracted twice. The three extracts were combined and diluted to 20 % methanol in nanopure water (v/v) before application to solid phase extraction.

Both the filtrate containing dissolved microcystins and the extracts containing intracellular microcystins were applied to solid phase extraction (Waters Oasis HLB) for clean up and concentration. Cartridges were conditioned with 5 mL of methanol followed by 5 mL of nanopure water. Samples were loaded at < 10 mL per minute. Cartridges were rinsed with 5 mL of 10 % methanol in nanopure water (v/v), followed by 5 mL of 20 % methanol in nanopure water (v/v), and eluted with 100 % methanol +0.1 % trifluoroacetic acid (TFA) (v/v). Samples were evaporated to dryness under nitrogen at 40 °C and stored at −20 °C until the day they were to be analysed by HPLC-PDA. On the day of analysis, samples were re-dissolved in 30 % acetonitrile (ACN) in nanopure water (v/v).

To determine microcystins concentrations, samples from each laboratory and full-scale trial were first analysed by high performance liquid chromatography coupled to photodiode array detection (HPLC-PDA) followed by liquid chromatography coupled to mass spectrometry (LC-MS) to confirm that chromatographic peaks were microcystins. Peaks with UV spectra characteristic of microcystins (Lawton et al., 1994; Meriluoto and Spoof, 2005) were identified by HPLC-PDA at 238 nm (1.2 nm resolution) using the gradient detailed in Lawton et al. (1994), but with a maximum acetonitrile concentration of 100 %. The HPLC-PDA system used was a Waters Alliance 2695 instrumentation with PDA detector and an Atlantis T3 separation column (3 µm, 100 Å, 4.6×150 mm i.d.). Analysis of peaks was performed using Empower (version 2). The molecular masses of the microcystins were identified by LC-MS using an Agilent 1200 series capillary pump with a 6340 Ion Trap mass selective detector, using the same separation column as for the HPLC. Mass analysis was performed by electrospray ionisation in positive mode, and data analysis conducted using ChemStation (version 3.4).

Three peaks were apparent in the HPLC-PDA and LC-MS chromatograms which had both UV spectra and masses characteristic of microcystins (masses listed in International Organization for Standardization, 2005). These peaks, microcystin-LR, microcystin-FR and microcystin-WR, were identified within samples from both the laboratory and full-scale trials. Although it is possible that there were other microcystins present in the samples, these were the only toxins which could be conclusively identified, and were thus chosen to represent the characteristic behaviour of microcystins following H_2O_2 treatment within these studies. These three microcystin species were quantified using HPLC-PDA and a commercially available microcystin-LR standard (Sapphire Bioscience, Australia). Total microcystins concentrations were calculated as the sum of intracellular and dissolved cyanotoxins.

2.6 Data analyses

Statistical analyses were conducted using SigmaPlot (version 8.0). Standard errors were calculated for the first laboratory trial, but could not be calculated for the second laboratory trial or the full-scale trial, due to the lack of replication. In the full-scale trial, exponential curve fits were used to explain the behaviour of chl *a* and microcystins in the longer term. The apparent behaviour of phycocyanin fluorescence immediately following H_2O_2 treatment was described by a logarithmic growth model.

3 Results and discussion

3.1 Cyanobacterial dynamics

In most of the laboratory samples treated with H_2O_2, cyanobacterial chl *a* appeared to increase in the 24 h immediately following H_2O_2 treatment. A significant reduction in cyanobacterial chl *a* was not observed until one to three days following addition (Figs. 1c, 2e and g). This apparent increase in cyanobacteria was likely an artefact of chl-*a* measurement using the FluoroProbe. This spectrofluorometer estimates the total cell chl *a* by measuring the characteristic spectra of photosynthetic antenna pigments after excitation with visible light, and converting the pigment concentration to chl *a* equivalents (Beutler et al., 2002). Upon cyanobacterial cell lysis following H_2O_2 treatment, the pigment phycocyanin may be released to the extracellular, dissolved state. Recent work suggests that the release of phycocyanin may increase the apparent concentration of cyanobacterial chl *a* when measured using spectrofluorometry (Bastien et al., 2011; Moldaenke, 2009).

To confirm this, we performed a similar investigation to Moldaenke (2009) in our laboratory by sonicating cyanobacterial samples for seventy minutes to induce cell lysis. The cyanobacterial biomass of samples was measured in five minute intervals until no further increase in phycocyanin was observed. Results were normalised to an unsonicated control, and there was a maximum apparent chl *a* increase of 53 % following the release of phycocyanin to the dissolved phase (logarithmic growth, $R^2_{\text{adj}} = 0.81$, ANOVA, $P = 0.0002$, results not shown).

Phycocyanin is isomerised by high pH and urea concentrations (Berns and Maccoll, 1989), both common conditions in WSPs, and hence the dissolved pigment is not detected by spectrofluorometry within hours to days of cell lysis. This results in an apparent lag in cyanobacterial reduction following H_2O_2 addition. However, in our study cyanobacterial reduction certainly occurred following the addition of H_2O_2, indicated by visual observations of mass cell death.

The final concentrations of cyanobacterial chl *a* in the laboratory trials, several days after H_2O_2 treatment, were likely indicative of chl *a* concentrations contained within the remaining live cyanobacterial cells. However, measurements collected immediately following H_2O_2 treatment in all trials were probably heavily influenced by dissolved phycocyanin concentrations. This suggests that short-term measurements of antenna pigments may not reflect the overall behaviour of cyanobacterial colonies, particularly if blooms are collapsing from human intervention or natural means. This is an important consideration for water managers, as many technologies for determining algal biomass using chl *a* and phycocyanin fluorescence are currently being developed and commercialized (see for example Gregor and Marsalek, 2004; Izydorczyk et al., 2005; Richardson et al., 2010).

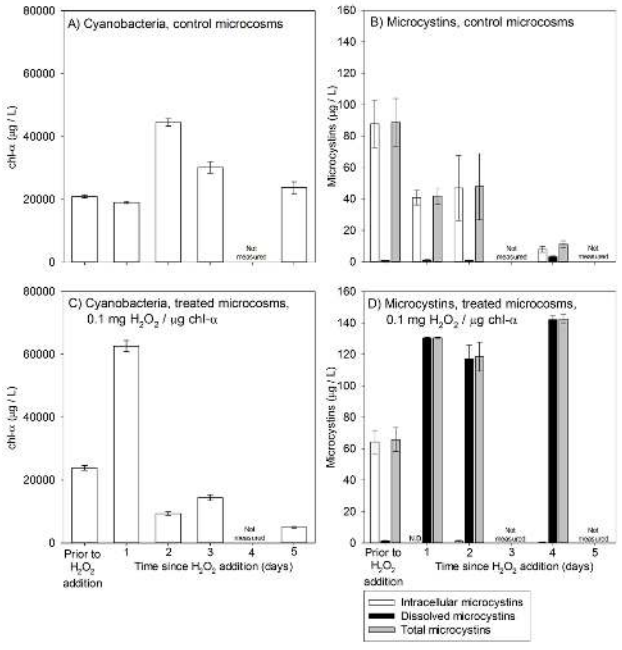

Fig. 1. Results from the first laboratory trial. **(A)** and **(B)** demonstrate cyanobacteria and microcystins dynamics in the control microcosms, whilst **(C)** and **(D)** demonstrate their behaviour in the treated microcosms. All microcystins concentrations are expressed as microcystin-LR equivalents. Error bars represent standard errors where $N = 2$.

In the field experiment, cyanobacteria at the pond outlet decreased significantly in the week following H_2O_2 treatment, and then exhibited exponential growth (ANOVA, $P < 0.01$) for the final three weeks of the monitoring period (Fig. 3a). This was probably due to the influx of cyanobacteria from WSPs earlier in the treatment train (notice the increasing concentration of cyanobacteria at the pond inlet in Fig. 3b), compounded by the environmental conditions favouring cyanobacterial abundance.

3.2 Microcystins dynamics

Total microcystins concentrations increased throughout the sampling period in the control and lowest H_2O_2 treated microcosms in the second laboratory trial, indicating that microcystins were produced in both control and treated microcosms (Fig. 2b and d). It has been suggested that stress conditions may favour the increased production of microcystins in cyanobacteria (Kurmayer, 2011; Wang et al., 2007), and that significant production can take place on the scale of hours (Wood et al., 2012). The rapid increase observed in this trial may have been stimulated by laboratory conditions and the addition of H_2O_2.

In the microcosms treated with ≥ 0.1 mg H_2O_2 μg^{-1} chl *a*, intracellular microcystins concentrations decreased and dissolved microcystins concentrations increased (Figs. 1d, 2f

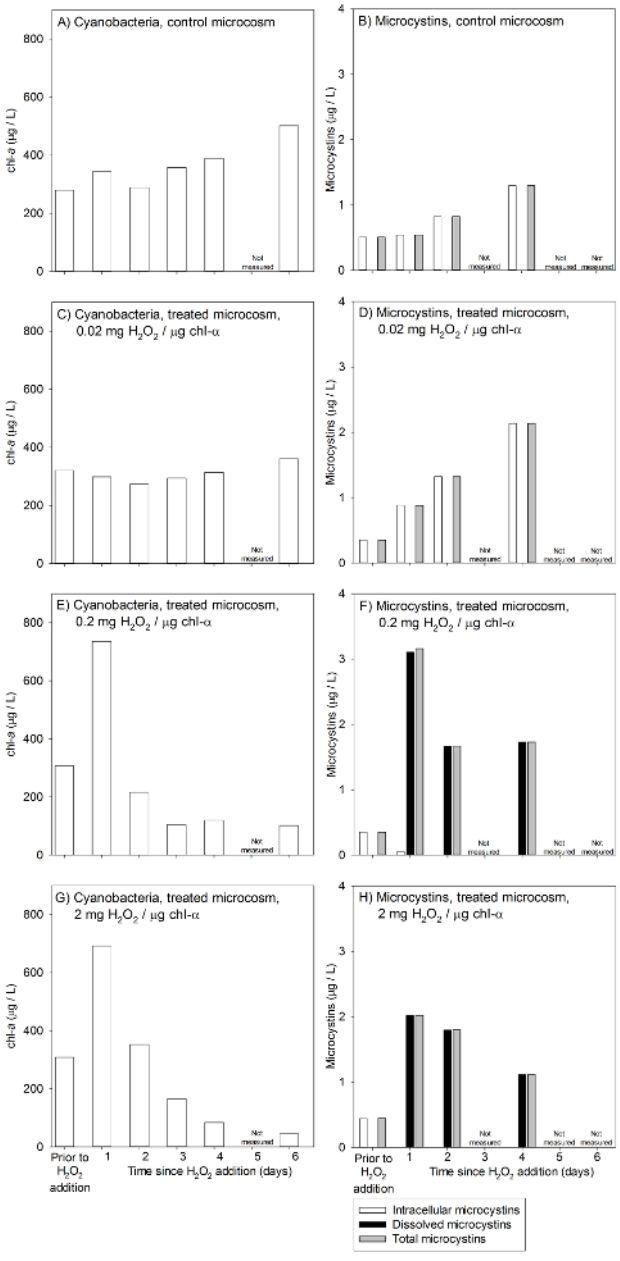

Fig. 2. Results from the second laboratory trial. **(A)** and **(B)** demonstrate cyanobacteria and microcystins dynamics in the control microcosms, whilst **(C)**, **(D)**, **(E)**, **(F)**, **(G)** and **(H)** demonstrate their behaviour in the treated microcosms. All microcystins concentrations are expressed as microcystin-LR equivalents.

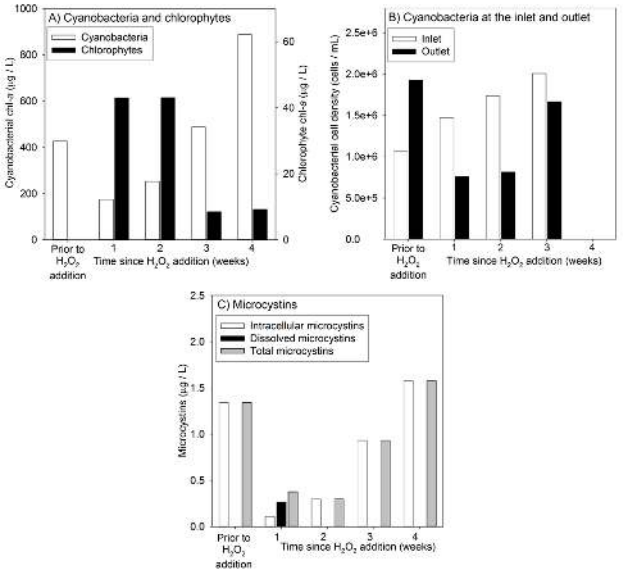

Fig. 3. Results from the Merredin waste stabilisation pond full-scale trial. Weekly averages are displayed in **(A)** and **(C)**. **(A)** demonstrates changes in biomass of cyanobacteria and Chlorophyta (measured using spectrofluorescence) at the pond outlet, **(B)** the behaviour of cyanobacteria (measured as cell density) at both the inlet and outlet and **(C)** the behaviour of intracellular and dissolved microcystins at the pond outlet. All microcystins concentrations are expressed as microcystin-LR equivalents.

and h). This indicates that where H_2O_2 was added at a concentration sufficient to induce cyanobacterial cell lysis, microcystins were released to the dissolved state. In the second laboratory trial (where H_2O_2 concentrations expressed as mass per unit volume were much smaller than in the first laboratory trial: the treated microcosms in laboratory trial 1 received $\sim 4\,g\,L^{-1}$ H_2O_2, whilst the treated microcosms in laboratory trial 2 received only 0.1–1 $g\,L^{-1}$ H_2O_2), dissolved microcystins concentrations decreased in the days following their release from cyanobacterial cells, indicating that some microcystins degradation was occurring within the water column (Fig. 2f and h). Hydrogen peroxide degrades within hours of addition (Cooper et al., 1994; Drábková et al., 2007), but dissolved microcystins will only be oxidised by H_2O_2 whilst it is still present within the water column, hence degradation by H_2O_2 was not the sole mechanism by which microcystins were decreased.

The degradation of microcystins by processes other than H_2O_2 addition is further illustrated by the results of the control microcosms of the first laboratory trial (Fig. 1b). A reduction in microcystins concentration within live cyanobacterial cells has not been observed in previous work, and extensive studies into the degradation of microcystins suggest that they are primarily degraded within the water column (World Health Organization, 2003). This suggests that in the control microcosms of the first laboratory trial (Fig. 1b) significant dissolved microcystins degradation occurred without H_2O_2 addition.

Bacterial degradation is a rapid natural mechanism for the removal of dissolved microcystins from the environment (Harada and Tsuji, 1998). It is likely that the significant decrease in dissolved microcystins in the control microcosms was due to the presence of natural bacteria in the WSP assemblage (Ho et al., 2010). The death of indigenous

microcystins-degrading bacteria following the addition of H_2O_2 may explain why the degradation of dissolved microcystins in the treated microcosms of the first laboratory trial was minimal compared to controls. This may also explain why dissolved microcystins were degraded in treated microcosms of the second laboratory trial, where a lower H_2O_2 concentration (compared to the first laboratory trial) may not have killed all microcystins-degrading bacteria. Determining the response of microcystins-degrading bacteria to H_2O_2 addition requires further investigation.

In the full-scale trial intracellular microcystins were released to the dissolved phase immediately following H_2O_2 addition, and these dissolved microcystins were then degraded throughout the week following H_2O_2 addition (Fig. 3c). Although the processes occurring in the laboratory were likely also occurring in the field, the full-scale trial would have introduced further potential mechanisms for microcystins degradation, including the presence of UV radiation, which catalyses the production of hydroxyl and hydroperoxyl radicals from H_2O_2, resulting in a high degree of microcystins degradation in the hours following H_2O_2 addition (Glaze et al., 1987). Microcystins can also be destroyed by UV radiation through isomerisation, and photosensitised transformation in the presence of humic substances and pigments (Song et al., 2007; Tsuji et al., 1994; Welker and Steinberg, 1999, 2000). In the full-scale trial, there would also have been a significantly higher loading of sediment than in the laboratory trials, where wastewater was collected from the surface of ponds. Adsorption by sediments, and subsequent biodegradation, may have accelerated the reduction of microcystins in the full-scale trial compared to the laboratory trials (Harada and Tsuji, 1998; Liu et al., 2008). Biodegradation, UV radiation and adsorption may all have played a role in the longer term reduction of microcystins from the dissolved phase, and may have accelerated their reduction compared to the smaller scale trials under controlled laboratory conditions.

These studies indicate that the addition of H_2O_2 results in the release of microcystins to the dissolved phase but that a combination of H_2O_2 and natural mechanisms then degrade these dissolved toxins. This means that under field conditions the addition of H_2O_2 leads to a significant reduction in total microcystins, concurrently reducing the toxicity of the waterway. This suggests that H_2O_2 addition under environmental conditions may be suitable for reducing cyanotoxin concentrations in WSP assemblages. However, given the large variation between the laboratory and field results, it is imperative that further studies into the use of H_2O_2 are conducted in full-scale WSPs, particularly regarding the effects of H_2O_2 on microcystins-degrading bacteria.

3.3 Cyanobacteria and Chlorophyta coupling in the full-scale trial

The full-scale field trial was conducted for significantly longer than the laboratory trials, water was continuously flowing into the maturation pond, and the temperature could not be controlled. Hence the results of the full-scale trial demonstrate the longer-term behaviour of the cyanobacterial and phytoplankton assemblage following H_2O_2 addition, including influence of inflows and diurnal temperature fluctuations, which could not be investigated under laboratory conditions.

Prior to H_2O_2 treatment at the full scale, Chlorophyta, predominantly of the genera *Chlamydomonas*, *Closterium*, *Scenedesmus*, *Monoraphidium*, and *Pandorina* were present at low concentrations, particularly compared to cyanobacteria, at both the inlet and outlet of the Merredin maturation WSP (Chlorophyta ~ 5000 cells mL^{-1}, cyanobacteria $\sim 2\,000\,000$ cells mL^{-1}, data not shown). Chlorophyta are beneficial to WSPs, as they produce the oxygen required for wastewater treatment without significantly impacting upon treatment efficiency or posing a risk due to toxicity, unlike cyanobacteria. In the full-scale trial, the concentration of Chlorophyta at the outlet increased significantly in the two weeks following treatment with H_2O_2. Chlorophyta then decreased significantly in the third week following H_2O_2 treatment (Fig. 3a).

Several previous studies have determined that eukaryotic phytoplankton are less susceptible to H_2O_2 toxicity than prokaryotic cyanobacteria (e.g. Barrington and Ghadouani, 2008; Drábková et al., 2007). In the Merredin WSP, following H_2O_2 treatment, a large proportion of cyanobacteria was rapidly eliminated by H_2O_2, allowing the Chlorophyta a competitive advantage, and resulting in an increase in Chlorophyta abundance (Fig. 3a). However, in the subsequent weeks, cyanobacteria and intracellular microcystins exhibited exponential growth (ANOVA, $P < 0.01$), with a resulting decrease in Chlorophyta concentration (Fig. 3). Cyanobacteria often dominate stratified reservoirs due to their ability to regulate their buoyancy, hence outcompeting other algae for light, and shading other organisms from incident radiation (Passarge et al., 2006). Whilst removing cyanobacteria from WSPs may allow the return of beneficial algal species, the continuing re-establishment of a pond with cyanobacteria (Fig. 3b) can rapidly reduce Chlorophyta abundance by competition. The phytoplankton assemblage of the WSP then returns to cyanobacterial dominance; probably with a resulting increase in microcystins (as observed in Fig. 3).

4 Conclusions

Although H_2O_2 may be a short-term strategy for the removal of cyanobacteria and microcystins, processes within the WWTP which contribute to rapid cyanobacterial growth must also be assessed, so that re-establishment of the cyanobacterial colony does not occur soon after algicidal treatment. It is thus important to consider whether it would be beneficial to remove cyanobacteria and microcystins earlier in wastewater treatment, such that the following WSPs in the treatment train are not infected by a continuous inflow of cyanobacteria.

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