Behaviour of cyanobacterial bloom material following coagulation and/or sedimentation

Lionel Ho, Albane Barbero, Jennifer Dreyfus, David R. Dixon, Feng Qian, Peter J. Scales and Gayle Newcombe

ABSTRACT

The global increase in detection of cyanobacteria and their metabolites has prompted greater emphasis in optimizing water treatment options for their effective removal. In particular, conventional coagulation and clarification processes have been shown to be effective in removing whole cyanobacterial cells, and consequently a majority of the metabolites. However, the resultant cyanobacterial-laden sludge is often not closely monitored in treatment plants. This study has shown that the sludge supernatant derived from the coagulation/sedimentation of a range of natural cyanobacterial blooms contained extremely high concentrations of metabolites (up to ~8,000 ng L^{-1} of geosmin and ~90 μg L^{-1} of microcystins), which was attributed to cell lysis. Furthermore, the fate of the metabolites in the sludge supernatants differed with geosmin shown to be biodegradable, while the microcystins were released at different stages with some variants shown to be more persistent. The release and biodegradation of the metabolites followed pseudo-first-order kinetics with rate constants comparable to experiments using laboratory-cultured cyanobacteria. A key finding from this study was that the identification of cyanobacterial cell type is critical in making informed operational decisions, as not only did the cells behave differently within the sludge, but also the released metabolites behaved quite differently within the time frames studied.

Key words | coagulation, cyanobacteria, cylindrospermopsin, geosmin, microcystin, sludge

Lionel Ho (corresponding author)
Albane Barbero
Jennifer Dreyfus
Gayle Newcombe
Australian Water Quality Centre, SA Water Corporation,
250 Victoria Square,
Adelaide,
SA 5000,
Australia
E-mail: *lionel.ho@sawater.com.au*

David R. Dixon
Feng Qian
Peter J. Scales
Department of Chemical & Biomolecular Engineering,
The University of Melbourne,
VIC 3010,
Australia

INTRODUCTION

The impacts of cyanobacteria on water and wastewater treatment processes have become more closely scrutinized in recent times due to increased detection of these organisms in water bodies; a consequence that some believe is due to the impacts of climate change and global warming (Paerl *et al*. 2011). It is well established that these organisms have the propensity to produce metabolites, which can be recalcitrant to conventional treatment processes and, as such, have the ability to impair the quality of water from an aesthetic and human health perspective.

While there is an abundance of studies in the literature that have assessed various treatment options for the removal of cyanobacteria and their metabolites, one important area where there are knowledge gaps is the fate of cyanobacterial-laden sludge derived from coagulation and/or clarification processes. The coagulation process is one of the key treatment barriers for mitigating cyanobacteria and many studies have demonstrated its effectiveness for this purpose (Chow *et al*. 1998, 1999; Drikas *et al*. 2001; Ma *et al*. 2007). However, one of the forgotten processes proceeding coagulation is the management of the resultant cyanobacterial-laden sludge, which can harbour significant numbers of cyanobacterial cells. If this sludge is not managed appropriately, there is potential for cell lysis, resulting in high concentrations of undesirable metabolites being released into the sludge supernatant. This can be an

doi: 10.2166/aqua.2013.137

issue if this supernatant (containing such metabolites) is returned to the head of treatment plants, particularly since conventional coagulation, clarification and filtration processes have been documented to be ineffective for the removal of extracellular metabolites (Chow *et al.* 1998, 1999; Mouchet & Bonnélye 1998; Newcombe & Nicholson 2004).

A recent study has shown that cyanobacterial-laden sludge can release significant concentrations of metabolites into supernatants after 3 d, and that the cells remained viable for up to 7 d (Ho *et al.* 2012). That study was conducted using laboratory-cultured species of *Anabaena circinalis* and *Cylindrospermopsis raciborskii*. Literature is sparse when determining whether natural blooms of cyanobacteria behave similarly to laboratory-cultured cyanobacteria due to the different matrices in which the cyanobacteria proliferate. Dixon *et al.* (2011) treated a natural bloom material using an integrated system comprising coagulation, powdered activated carbon and ultrafiltration; however, they could not make any definitive comparisons with laboratory-cultured cells due to the higher cell numbers of the natural bloom material. Consequently, there is a need to ascertain whether coagulated sludge, containing natural cyanobacterial bloom material, behaves similarly to sludge containing laboratory-cultured cyanobacteria. In addition, the rate of metabolite release and its subsequent degradation in such sludge is yet to be determined.

The aim of this study was to examine the behaviour of cyanobacteria and their metabolites in coagulated sludge derived from natural blooms. In particular, this study focused on the rate of metabolite release and degradation while in the sludge supernatant. Efforts were also made to compare the findings with laboratory-cultured cyanobacteria.

EXPERIMENTAL PROCEDURES

Cyanobacterial bloom material

Between January and March 2011, five significant cyanobacterial blooms occurred in various water sources in South Australia. Three of the sources were surface waters that supplement drinking water treatment plants while the other two were wastewater lagoons (see Table 1).

Laboratory-cultured cyanobacteria

Three cyanobacteria were used in this study: *A. circinalis* (reference strain ANA188B, AWQC, Adelaide SA, Australia), *C. raciborskii* (reference strain CYP011K, AWQC, Adelaide SA, Australia) and *Microcystis aeruginosa* (reference strain MIC388, AWQC, Adelaide SA, Australia). The strain of *A. circinalis* produced geosmin while the strain of *C. raciborskii* produced cylindrospermopsin (CYN). The strain of *M. aeruginosa* produced three microcystin variants, microcystin-LR, -LA and -RR (MCLR, MCLA and MCRR, respectively). The cyanobacteria were cultured in ASM-1 medium according to the methods of Gorham *et al.* (1964). The cultures were incubated at 20 °C under 12 h rotating light darkness flux at light intensity of 70 μmol s^{-1} m^{-2}.

Samples for cyanobacterial enumeration (by microscopy using a Nikon Eclipse 50i microscope) were treated with Lugol's iodine, pressurized to 750 kPa for 2 min (900 kPa for 2 min. for *M. aeruginosa*) to collapse gas vesicles, then counted in a gridded Sedgewick-Rafter chamber at 200× magnification using methods described previously (Brookes *et al.* 1994). Tests for cell viability were conducted (on a subsample in the absence of Lugol's iodine) by assessing the

Table 1 | Blooms of cyanobacteria in South Australian water sources from January–March 2011

Water source and type	Cyanobacteria present	Metabolites produced/detected
Kangaroo Creek/drinking water (January 2011)	*Anabaena circinalis* (3.0×10^6 cells mL^{-1})	Geosmin
Mt Bold/drinking water (January 2011)	*Anabaena circinalis* (1.8×10^5 cells mL^{-1})	Geosmin
Murray Bridge/drinking water (March 2011)	*Pseudanabaena* (9.7×10^5 cells mL^{-1})	MIB
Angaston/wastewater lagoon (March 2011)	*Microcystis flos aquae* (5.2×10^5 cells mL^{-1})	Microcystin-RR and -YR
Bolivar/wastewater lagoon (March 2011)	*Microcystis aeruginosa* (cell count not determined)	Microcystin-RR, -YR and -LR

autofluorescence (chlorophyll *a*) of the cells with a mercury lamp using a WG2 setup on the microscope: the wavelength of light (green) used to excite the sample was 480–550 nm, which produced a red emission spectrum.

Water studied

The laboratory-cultured cyanobacteria were re-suspended in Myponga Reservoir water at a cell number of 300,000 cells mL^{-1} for *M. aeruginosa* and *C. raciborskii*, and 900,000 cells mL^{-1} for *A. circinalis*. This South Australian drinking water source frequently experiences blooms of geosmin-producing *A. circinalis* and had the following characteristics at the time of this study: turbidity = 1 NTU, dissolved organic carbon (DOC) = 12.3 mg L^{-1}, UV absorbance at 254 nm = 0.474 cm^{-1}, pH = 7.6.

Coagulation/sedimentation experiments

Coagulation experiments were conducted on the natural bloom samples and laboratory-cultured cyanobacterial samples (in Myponga Reservoir water) at room temperature (25 °C) to generate cyanobacterial-laden sludge using a method similar to Chow *et al.* (1999). Briefly, an Ultra Torque BDC 1850 overhead stirrer fitted with a 75 mm × 25 mm straight blade impeller (Caframo, Canada) was employed for agitation in custom-made Perspex gator jars containing 5 L of cyanobacterial-spiked waters. Aluminium sulfate (alum) as $Al_2(SO_4)_3.18H_2O$ (80 or 100 mg L^{-1}) was added while stirring at 200 rpm ($G = 480\ s^{-1}$). After 1 min, the speed was reduced to 20 rpm ($G = 18\ s^{-1}$) for 14 min. The samples were allowed to sediment (without agitation) for 15 min. For the Murray Bridge and Bolivar natural bloom samples, no coagulation was employed; rather, the samples were allowed to sediment overnight. After sedimentation, samples (between 100 and 200 mL) were taken from the supernatants at regular intervals (every 1–4 days) to determine metabolite concentrations. These samples were immediately filtered through a GFC filter (Whatman, UK) to remove cellular material, then analysed for extracellular (dissolved) metabolites. In selected experiments, samples were also taken from the resultant sludge to determine cell viability.

Analyses

Samples for geosmin analyses were pre-concentrated using a solid phase microextraction syringe fibre (Supelco, Australia) and analysed on a 7890 Gas Chromatograph System with 5975C VL Series Mass Selective Detector (Agilent Technologies, Australia) against quantified labelled internal standards (Ultrafine Chemicals, UK). Full details of this method have been documented by Graham & Hayes (1998).

Prior to high performance liquid chromatographic (HPLC) analyses, microcystins and CYN were concentrated from waters by solid phase extraction using methods described previously by Nicholson *et al.* (1994) and Metcalf *et al.* (2002), respectively. An Agilent 1100 series HPLC system comprising of a quaternary pump, autosampler and photodiode array detector (Agilent Technologies, Australia) was employed for their analyses. Full details of the methods have been described previously (Ho *et al.* 2011).

Rate constant determination

The release and degradation of the metabolites was modelled as a pseudo-first-order reaction based on a simplified Michaelis–Menten equation (Schmidt *et al.* 1985; Anderozzi *et al.* 2006; Daly *et al.* 2007; Ho *et al.* 2007). These previous studies have determined that the degradation of such organic compounds (which are utilized as secondary substrates) followed pseudo-first-order kinetics.

RESULTS AND DISCUSSION

Cyanobacterial bloom material

Table 1 lists the cyanobacteria and the respective metabolites detected in the natural bloom samples from the various water sources. Experiments utilizing natural cyanobacterial blooms are sparse, mainly due to the sporadic nature of the blooms themselves. However, our research team was opportunistic in an approach to sampling the blooms when they occurred and conducting experiments within a timely manner (on the same day). Coagulation experiments were conducted on all natural bloom samples except for Bolivar and Murray Bridge, where the

cyanobacteria were allowed to sediment unassisted. Preliminary coagulation experiments determined the optimum alum doses for the natural bloom material in Angaston to be 100 and 80 mg L^{-1} for Kangaroo Creek and Mt Bold (results not shown). Briefly, the optimum doses were selected based not only on removal of cells, but also on removal of other water-quality parameters including turbidity, DOC and UV absorbance (see method and rationale in Ho *et al.* 2012).

After coagulation and/or sedimentation of the cyanobacterial cells, samples were taken from the resultant supernatants for a period of up to 30 d to ascertain the fate of the metabolites. Figure 1 shows the concentrations of the dissolved metabolites as a function of time in the supernatants of the respective natural bloom material. The geosmin and MIB concentrations initially increased within the first 5–10 d in the Kangaroo Creek, Mt Bold and Murray Bridge samples, after which they slowly decreased to below detection between 16 and 22 d (Figures 1(a)–1(c)). These observations are indicative of the release of geosmin and MIB from the cells, followed by subsequent biodegradation. Both geosmin and MIB have been shown to be biodegradable in these water sources, lending support to this contention (Ho *et al.* 2007).

The rate of release and degradation of geosmin and MIB followed pseudo-first-order kinetics with the calculated rate constants shown in Table 2. The rate of geosmin release was more rapid than its subsequent degradation, while the opposite was apparent for MIB. The more rapid release of geosmin may be due to its physicochemical properties, in particular its volatility and solubility (in water). The Henry's law constant and aqueous solubility of geosmin have been reported to be 6.7×10^{-5} atm m^3 mol^{-1} and 150 mg L^{-1}, respectively, with the corresponding values for MIB being 5.8×10^{-5} atm m^3 mol^{-1} and 195 mg L^{-1} (Pirbazari *et al.* 1992). It should also be noted that the rate of metabolite release may also be impacted by factors including the cyanobacterial cell type (speciation and morphology) and the growth phase (physiological state) of the cells. For example, studies have shown that for *C. raciborksii* a substantial proportion of CYN is extracellular (up to 90% in some cases) compared with the microcystins from *Microcystis* sp., which are almost entirely intracellular (Park *et al.* 1998; Griffiths & Saker 2003; Falconer 2005; Graham *et al.* 2008).

In contrast to geosmin and MIB, differences were observed with the microcystins in the Angaston and Bolivar samples (Figures 1(d) and 1(e), respectively). In the Angaston bloom material, two microcystin variants were produced, MCRR and microcystin-YR (MCYR), and both of these variants behaved differently. While MCRR exhibited a similar pattern to geosmin and MIB (where an initial increase in concentration was followed by a decrease, attributed to biodegradation), the fate of MCYR was markedly different, where its release from the cells did not occur until day 13, after which time the concentration increased up to ~90 μg L^{-1} on day 22. The slower release of MCYR compared with MCRR may be due to the molecular structures of the variants, which have previously been shown to influence their adsorption onto activated carbon; in particular, MCRR has been shown to be more easily adsorbed than MCYR (Cook & Newcombe 2002; Ho *et al.* 2011). MCRR is more hydrophilic than MCYR and has a net neutral charge between pH 6.0 and 8.5 compared with MCYR, which has a net charge of –1 in the same pH range (Fastner *et al.* 1998; Ho *et al.* 2011). These factors result in the differences in their adsorption and hence may also account for the differences in their release from cells. The speciation and physiological nature of the cells may also impact on the release of the microcystin variants.

In the Bolivar sample, trace levels of MCLR and MCRR were produced (<0.5 μg L^{-1}), with no observable release of either variant. However, MCYR was released from the sample after 8 d with the concentration reaching a peak of ~70 μg L^{-1} on day 14, after which time the concentration decreased to below analytical detection on day 21. This decrease was attributed to biodegradation as microcystins have previously been shown to be biodegradable in this water source (Ho *et al.* 2010).

The MCRR and MCYR release rates also followed pseudo-first-order kinetics with the rate constants listed in Table 2. The rates of MCYR release were the same order of magnitude as geosmin and MIB, with more rapid release observed in the Angaston sample compared with Bolivar. The rate of MCYR degradation in the Bolivar sample was an order of magnitude greater than a previous study utilizing water from Bolivar (Ho *et al.* 2010). However, that study utilized water sampled downstream of the lagoons, more specifically, after tertiary treatment comprising dissolved

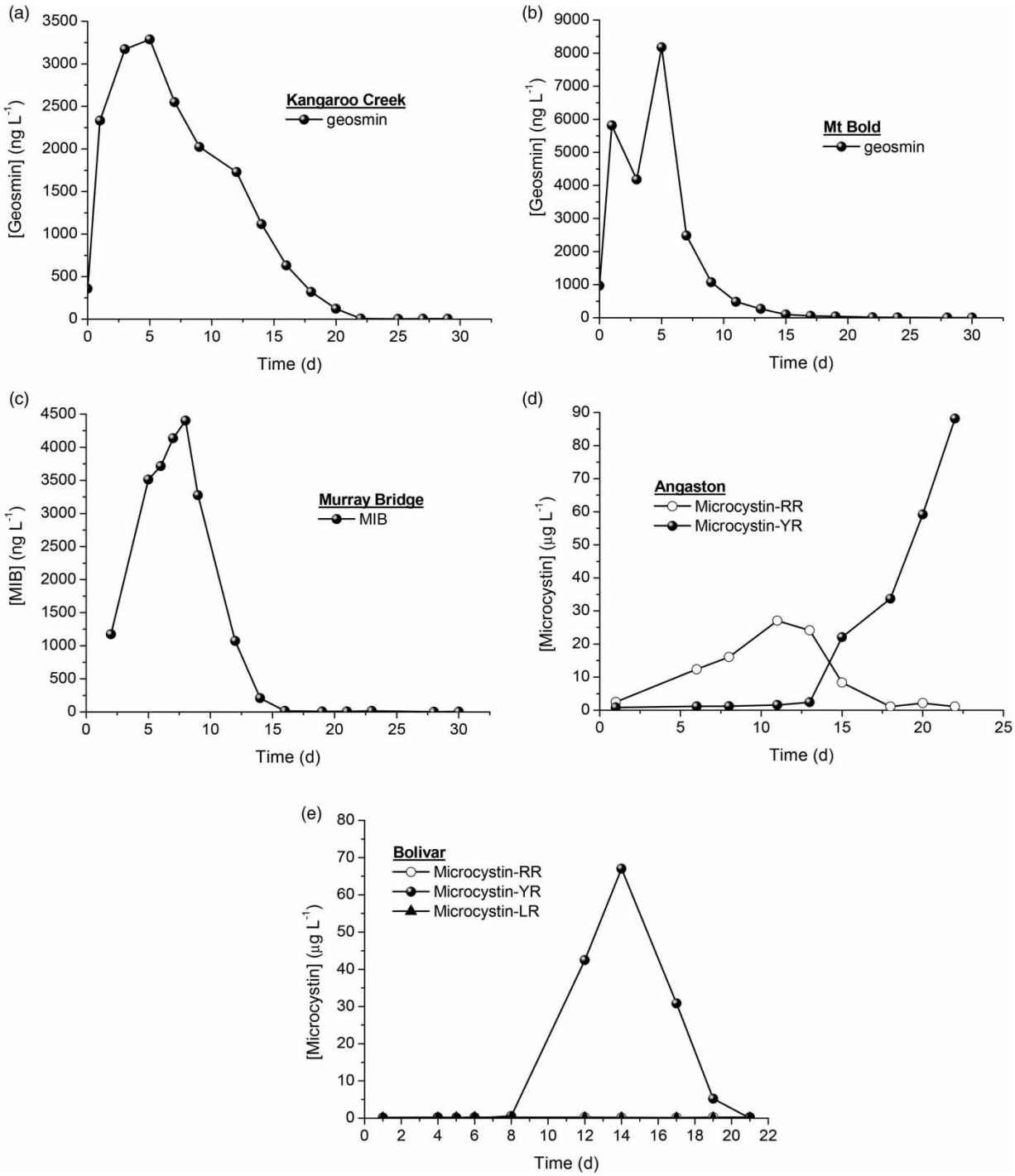

Figure 1 | Fate of dissolved metabolites in cyanobacterial-laden sludge following coagulation and/or sedimentation: (a) geosmin in Kangaroo Creek; (b) geosmin in Mt Bold; (c) MIB in Murray Bridge; (d) microcystin-RR and -YR in Angaston; (e) microcystin-RR, -YR and -LR in Bolivar.

air flotation and filtration (DAFF). Consequently, numbers of the degrading organisms would be lower in abundance in the DAFF treated water, since coagulation processes have been shown to remove bacterial numbers (Hoefel *et al.* 2005). This results in a lower rate of degradation as studies have demonstrated that the degradation rate is

Table 2 | Pseudo-first-order rates of metabolite release and degradation within the cyanobacterial-laden sludge from natural bloom material

	k (h^{-1})	
Metabolite	**Release**	**Degradation**
Geosmin (Kangaroo Creek)	1.9×10^{-2} (R^2 0.70)	8.4×10^{-3} (R^2 0.88)
Geosmin (Mt Bold)	1.8×10^{-2} (R^2 0.98)	1.4×10^{-2} (R^2 0.98)
MIB (Murray Bridge)	3.3×10^{-3} (R^2 0.98)	2.7×10^{-2} (R^2 0.91)
Microcystin-RR (Angaston)	1.0×10^{-2} (R^2 0.94)	1.3×10^{-2} (R^2 0.98)
Microcystin-YR (Angaston)	1.7×10^{-2} (R^2 0.94)	–
Microcystin-YR (Bolivar)	3.6×10^{-3} (R^2 0.98)	2.0×10^{-2} (R^2 0.78)

dependent upon the number of the degrading organisms (Hoefel *et al.* 2009; Ho *et al.* 2010).

Laboratory-cultured cyanobacteria

Similar coagulation and sedimentation experiments were conducted using laboratory-cultured cyanobacteria, more specifically, a geosmin-producing *A. circinalis*, a microcystin-producing *M. aeruginosa* and a CYN-producing *C. raciborskii*. Figure 2 shows the fate of the metabolites after coagulation and sedimentation of the three cyanobacteria. One water (Myponga Reservoir water) was selected for these experiments to allow for a direct comparison between the studied cyanobacteria and their respective metabolites. It is evident from

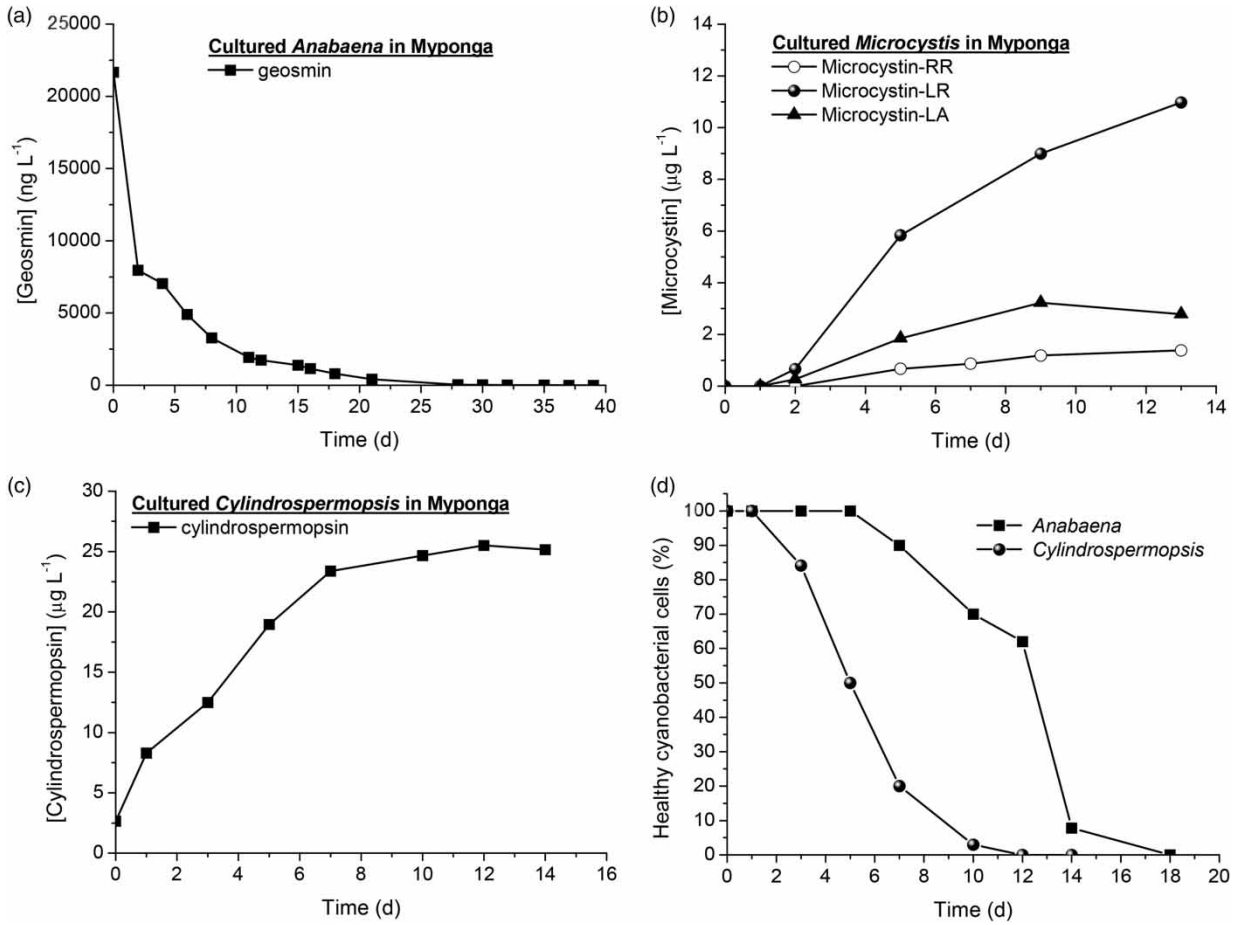

Figure 2 | Release of metabolites from cultured cyanobacterial-laden sludge following coagulation of Myponga Reservoir water: (a) geosmin from *A. circinalis* at 900,000 cells mL^{-1} following alum coagulation at 100 mg L^{-1}; (b) microcystin-RR, -LR and -LA from *M. aeruginosa* at 300,000 cells mL^{-1} following alum coagulation at 80 mg L^{-1}; (c) cylindrospermopsin from *C. raciborskii* at 300,000 cells mL^{-1} following alum coagulation at 80 mg L^{-1}; (d) percentage of healthy *A. circinalis* and *C. raciborskii* cells in sludge as a function of time following alum coagulation.

Figure 2 that each of the cyanobacteria behaved differently in terms of the release of the metabolites and their subsequent fate. All the geosmin was extracellular after coagulation and sedimentation (Figure 2(a)), the concentration of which decreased with time. This decrease was attributed to biodegradation, similar to the results from the natural bloom samples. In order to provide irrefutable evidence that the loss of geosmin was due to biodegradation, an additional experiment was conducted where geosmin was dosed into Myponga Reservoir water and compared with a parallel experiment conducted in sterilized Myponga Reservoir water (Figure 3). The lack of geosmin loss in the sterilized experiment confirmed that the loss in the unsterilized Myponga Reservoir water was due to biodegradation and not some other physical processes.

In contrast to geosmin, CYN and the microcystins (MCRR, MCLR and MCLA) were slowly released (from *C. raciborskii* and *M. aeruginosa*, respectively) after coagulation and sedimentation with no losses of either toxins within the time frame of the experiments (Figures 2(b) and 2(c)). This highlights the different behaviour of the three cyanobacterial species and their metabolites and indicates that approaches in managing such organisms in sludge supernatants should take into consideration the type of cyanobacteria present.

Figure 2(d) shows the viability of *C. raciborskii* and *A. circinalis* in the alum sludge as a function of time (*M. aeruginosa* viability was not evaluated). The decrease in the viability of both cyanobacterial cell types (by assessment of their autofluorescence) provides some evidence that the release of the metabolites (geosmin and CYN) was due to the diminishing health of the cells, and consequently cell lysis. A previous study has shown *A. circinalis* cells remained healthy (>90%) up to a period of 7 d in alum sludge (Ho *et al.* 2012), similar to the findings of this study; however, after 7 d in this study, the cells rapidly lose viability, with the majority of cells shown to be non-viable by day 18. Interestingly, *C. raciborskii* appeared to lose viability more rapidly than *A. circinalis* in the alum sludge with complete loss of viability by day 12. This suggests that *A. circinalis* cells are more robust than *C. raciborskii* cells in alum sludge, a finding which has only been previously anecdotally inferred (Chiswell *et al.* 1999; Griffiths & Saker 2003; Ho *et al.* 2012).

While a direct comparison could not be made between the natural bloom material with laboratory-cultured material (i.e. the same cell type in the same water), there were still some similarities. For example, geosmin was shown to be biodegradable in the supernatants of the natural bloom and laboratory-cultured materials. Furthermore, the biodegradation rate of geosmin in Myponga sludge supernatant (see Table 3) was similar to that of the Kangaroo Creek sample. The only other metabolite where a comparison could be made between the natural bloom material with laboratory-cultured material was MCRR. Based on the rate constant data, more rapid release of MCRR was observed in the Angaston bloom material ($1.0 \times 10^{-2}\,h^{-1}$) compared with Myponga sludge ($3.7 \times 10^{-3}\,h^{-1}$) (see Tables 2 and 3). This is thought to be due to the different *Microcystis* species producing MCRR in the different samples, in particular, greater production of MCRR by the Angaston bloom

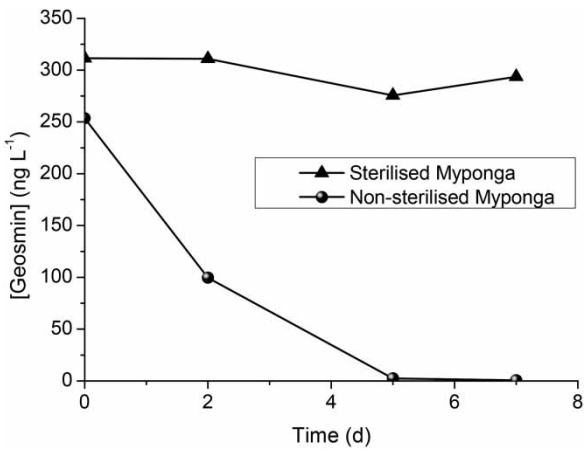

Figure 3 | Biodegradation of geosmin in Myponga Reservoir water.

Table 3 | Pseudo-first-order rates of metabolite release and degradation within the cyanobacterial-laden sludge from cultured material in Myponga Reservoir water

	k (h^{-1})	
Metabolite	**Release**	**Degradation**
Geosmin	–	6.3×10^{-3} (R^2 0.99)
Microcystin-RR	3.7×10^{-3} (R^2 0.86)	–
Microcystin-LR	1.5×10^{-2} (R^2 0.62)	–
Microcystin-LA	1.4×10^{-2} (R^2 0.71)	–
Cylindrospermopsin	1.1×10^{-2} (R^2 0.77)	

material (*M. flos aquae*) compared with the cultured *M. aeruginosa*, i.e. up to 27 μg L^{-1} compared with <2 μg L^{-1}, respectively. However, in the cultured *M. aeruginosa*, MCLR was produced at a higher concentration, ~11 μg L^{-1}, with a release rate (1.5×10^{-2} h^{-1}) comparable to that of MCRR from Angaston bloom material. The rates of MCLA and CYN release were also in the same order of magnitude as MCLR in cultured *M. aeruginosa*.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The results from this study showed that cyanobacterial-laden coagulant sludge can release copious amounts of metabolites into the supernatant. In most Australian treatment plants, such sludge is managed in lagoons where the process dewaters the sludge to assist in its disposal. However, of concern is the fact that in some cases the sludge supernatant from the lagoons can be recycled back to the head of the treatment plants, which can be problematic since extracellular cyanobacterial metabolites are not well removed by conventional coagulation, clarification and filtration processes. To date, little information exists in the public domain with respect to the fate of cyanobacterial metabolites in sludge supernatants derived from natural cyanobacterial blooms. This study provides some pertinent information, in particular:

- within 5 d, up to ~8,000 ng L^{-1} of geosmin was released from cells within alum sludge;
- the released geosmin (and MIB) was efficiently biodegraded to below analytical detection between 15 and 20 d;
- up to ~90 μg L^{-1} of MCYR was released from cells within alum sludge (up to 70 μg L^{-1} was released from sedimented cells in the absence of coagulant);
- the microcystins were released from cells at various stages (immediately for MCRR in Angaston; between 8 and 13 d for MCYR in Angaston and Bolivar), with instances where efficient biodegradation was observed (MCRR in Angaston; MCYR in Bolivar).

Such findings can assist water utilities in making informed operational decisions for the treatment/management of sludge supernatants. An important finding of the work was that identification of the cyanobacterial cell type is critical in making these decisions since the released metabolites behaved quite differently. For example, if geosmin was present, treatment plant operators may be able to prolong recycling the supernatant to the head of the plant (withholding times of up to 20 d, if operationally possible). However, this would obviously not be the case if microcystins were present, where additional treatment or management strategies (powdered activated carbon dosing or chlorination) would be required for the recycled supernatant. Further emphasizing the importance of identifying the cell type was the finding that *A. circinalis* cells were more robust than *C. raciborskii* cells in the coagulant sludge.

In some instances, results from the laboratory-cultured cyanobacteria were comparable to natural bloom material, in particular geosmin, where the pseudo-first-order rates of degradation were similar. The derived rate constants may be used not to only ascertain the half-lives of the metabolites, but also in mathematical models to simulate the fate of the metabolites under a range of conditions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Australian Research Council Linkage Grant (LP0883951) for funding this study.

REFERENCES

Anderozzi, R., Cesaro, R., Marotta, R. & Pirozzi, F. 2006 Evaluation of biodegradation kinetic constants for aromatic compounds by means of aerobic batch experiments. *Chemosphere* **62**, 1431–1436.

Brookes, J. D., Ganf, G. G. & Burch, M. D. 1994 The separation of forms of *Microcystis* from *Anabaena* in mixed populations by the application of pressure. *Aust. J. Mar. Freshwat. Res.* **45**, 863–868.

Chiswell, R. K., Shaw, G. R., Eaglesham, G., Smith, M. J., Norris, R. L., Seawright, A. A. & Moore, M. R. 1999 Stability of cylindrospermopsin, the toxin from the cyanobacterium, *Cylindrospermopsis raciborskii*: Effect of pH, temperature, and sunlight on decomposition. *Environ. Toxicol.* **14**, 155–161.

Chow, C. W. K., Drikas, M., House, J., Burch, M. D. & Velzeboer, R. 1999 The impact of conventional water treatment processes on cells of the cyanobacterium *Microcystis aeruginosa*. *Water Res.* **33**, 3253–3262.

Chow, C. W. K., House, J., Velzeboer, R. M. A., Drikas, M., Burch, M. D. & Steffensen, D. A. 1998 The effect of ferric chloride flocculation on cyanobacterial cells. *Water Res.* **32**, 808–814.

Cook, D. & Newcombe, G. 2002 Removal of microcystin variants with powdered activated carbon. *Water Sci. Technol. Water Supply* **2**, 201–207.

Daly, R. I., Ho, L. & Brookes, J. D. 2007 Effect of chlorination on *Microcystis aeruginosa* cell integrity and subsequent microcystin release and degradation. *Environ. Sci. Technol.* **41**, 4447–4453.

Dixon, M. B., Richard, Y., Ho, L., Chow, C. W. K., O'Neill, B. K. & Newcombe, G. 2011 A coagulation-powdered activated carbon-ultrafiltration – Multiple barrier approach for removing toxins from two Australian cyanobacterial blooms. *J. Hazard. Mater.* **186**, 1553–1559.

Drikas, M., Chow, C. W. K., House, J. & Burch, M. D. 2001 Using coagulation, flocculation and settling to remove toxic cyanobacteria. *J. Am. Water Works Assoc.* **93**, 100–111.

Falconer, I. R. 2005 *Cyanobacterial Toxins in Drinking Water Supplies: Cylindrospermopsins and Microcystins*. CRC Press, Boca Raton, Florida, USA.

Fastner, J., Flieger, I. & Neumann, U. 1998 Optimised extraction of microcystins from field samples – a comparison of different solvents and procedures. *Water Res.* **32**, 3177–3181.

Gorham, P. R., McLachlan, J., Hammer, U. T. & Sivonen, W. K. 1964 Isolation and culture of toxic strains of *Anabaena flos-aquae*. *Mitt. Int. Ver. Limnol.* **15**, 796–804.

Graham, D. & Hayes, K. P. 1998 Application of solid phase microextraction for the analysis of off-flavours in water. In Proceedings of the WaterTECH Conference, April 26–29, Brisbane, Australia.

Graham, J. L., Loftin, K. A., Ziegler, A. C. & Meyer, M. T. 2008 *Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs*. US Geological Survey Scientific Investigations Report, 2008–5038.

Griffiths, D. J. & Saker, M. L. 2003 The Palm Island mystery disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Environ. Toxicol.* **18**, 78–93.

Ho, L., Dreyfus, J., Boyer, J., Lowe, T., Bustamante, H., Duker, P., Meli, T. & Newcombe, G. 2012 Fate of cyanobacteria and their metabolites during water treatment sludge management processes. *Sci. Total Environ.* **424**, 232–238.

Ho, L., Hoefel, D., Bock, F., Saint, C. P. & Newcombe, G. 2007 Biodegradation rates of 2-methylisoborneol (MIB) and geosmin through sand filters and in bioreactors. *Chemosphere* **66**, 2210–2218.

Ho, L., Hoefel, D., Palazot, S., Sawade, E., Newcombe, G., Saint, C. P. & Brookes, J. D. 2010 Investigations into the biodegradation of microcystin-LR in wastewaters. *J. Hazard. Mater.* **180**, 628–633.

Ho, L., Lambling, P., Bustamante, H., Duker, P. & Newcombe, G. 2011 Application of powdered activated carbon for the adsorption of cylindrospermopsin and microcystin toxins from drinking water supplies. *Water Res.* **45**, 2954–2964.

Hoefel, D., Adriansen, C., Bouyssou, M., Saint, C. P., Newcombe, G. & Ho, L. 2009 Development of an *mlrA* gene-directed TaqMan PCR assay for quantitative assessment of microcystin-degrading bacteria within water treatment plant sand filter biofilms. *App. Environ. Microbiol.* **75**, 5167–5169.

Hoefel, D., Monis, P. T., Grooby, W. L., Andrews, S. & Saint, C. P. 2005 Profiling bacterial survival through a water treatment process and subsequent distribution system. *J. Appl. Microbiol.* **99**, 175–186.

Ma, J., Lei, G. & Fang, J. 2007 Effect of algae species population structure on their removal by coagulation and filtration processes – a case study. *J. Water Supply Res. Technol.* **56**, 41–54.

Metcalf, J. S., Beattie, K. A., Saker, M. L. & Codd, G. A. 2002 Effects of organic solvents on the high performance liquid chromatographic analysis of the cyanobacterial toxin cylindrospermopsin and its recovery from environmental eutrophic waters by solid phase extraction. *FEMS Microbiol. Lett.* **216**, 159–164.

Mouchet, P. & Bonnélye, V. 1998 Solving algae problems: French expertise and world-wide applications. *J. Water Supply Res. Technol.* **47**, 125–141.

Newcombe, G. & Nicholson, B. 2004 Water treatment options for dissolved cyanotoxins. *J. Water Supply Res. Technol.* **53**, 227–239.

Nicholson, B. C., Rositano, J. & Burch, M. D. 1994 Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. *Water Res.* **28**, 1297–1303.

Paerl, H. W., Hall, N. S. & Calandrino, E. S. 2011 Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Sci. Total Environ.* **409**, 1739–1745.

Park, H.-D., Iwami, C., Watanabe, M. F., Harada, K.-I., Okino, T. & Hayashi, H. 1998 Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic *Microcystis* species in a hypertrophic lake, Lake Suwa, Japan (1991–1994). *Environ. Toxicol. Water Qual.* **13**, 61–72.

Pirbazari, M., Borow, H. S., Craig, S., Ravindran, V. & McGuire, M. J. 1992 Physical chemical characterization of five earthy-musty-smelling compounds. *Water Sci. Technol.* **25**, 81–88.

Schmidt, S. K., Simkins, S. & Alexander, M. 1985 Models for the kinetics of biodegradation of organic compounds not supporting growth. *Appl. Environ. Microbiol.* **50**, 323–331.

First received 8 November 2012; accepted in revised form 23 May 2013