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Identification and assessment of water quality risks associated with sludge supernatant recycling in the presence of cyanobacteria

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ABSTRACT

This study focussed on the fate of cyanobacteria cells and associated metabolites during the sludge management processes that follow the conventional drinking water treatment train. The topic is of importance, as the release of metabolites during sludge treatment may pose a risk to water quality if supernatant is recycled to the head of the plant. The study of the kinetics of cell damage and metabolite release into the supernatant is complicated by simultaneous and rapid natural removal processes. In this study, the release of organic material from cyanobacterial sludge was monitored simultaneously with secondary metabolites (Microcystins (MCs), cylindrosperopsin (CYN), and geosmin (GSM)) as an additional parameter to aid in understanding the range of processes occurring in sludge. Only GSM produced by Dolichospermum circinale was found to represent a low risk, as the compound is readily degraded. In contrast, the metabolites CYN and MC were shown to increase in concentration during simulated sludge treatment, suggesting that this could occur within full scale sludge treatment facilities with a range of cyanobacteria species, metabolites and water quality. A generic risk matrix was developed, incorporating the type of cyanobacteria, metabolite production, and the treatment processes available to water utilities for the mitigation of the identified risks.

Keywords: cyanobacteria, cyanotoxins, dissolved organic carbon (DOC), sludge storage, water treatment.

INTRODUCTION

Cyanobacteria are found in surface water sources world- wide and are the cause of a range of operational challenges in drinking water treatment, including increased coagulant demand and sludge treatment costs, filter bed clogging and reduced filter run times (Chen et al. 2009; Shen et al.2011; Ma et al. 2012). While these complications can be problematic, the major water quality issues associated with cyanobacteria are the secondary metabolites they

produce. The metabolites that have the greatest impact on aesthetic water quality are the compounds 2-methylisoborneol (MIB) and geosmin (GSM), which impart an earthy/musty flavor and odour to the water that can be detected by a sensitive consumer at levels as low as 5–10 ng/L (Ho et al. 2007). Of greater concern from a health perspective are the algal toxins, or cyanotoxins (Chorus & Bartram 2002). The concentrations of these compounds can be effectively reduced by techniques such as activated carbon adsorption and oxidation using chlorine or ozone (Newcombe et al. 2010). However, the most effective and simplest barrier in the treatment process can be the removal of intact cells by conventional treatment of coagulation, sedimentation and filtration (Drikas et al. 2001). In a healthy cyanobacteria bloom, the metabolites are mainly contained within the cyanobacteria (intracellular) and between 50 and 95% can be removed during the conventional treatment process (Chorus & Bartram 2002). While the process of coagulation is effective for the removal of intracellular metabolites, the resultant accumulation of the flocs within the treatment plant or in the sludge treatment facility provides a reservoir of toxins and taste and odour compounds that can potentially compromise finished water quality (Ho et al. 2012a, 2013; Zamyadi et al. 2013; Pestana et al.2016). In addition, cells that are remaining in suspension after coagulation can accumulate within the plant, even when relatively low numbers of cells enter in the raw water (Zamyadi et al.2012, 2013). Another common practice is recycling of supernatant from sludge treatment facilities back to the head of the plant. Two recent publications have highlighted the risk associated with this practice if cyanobacteria accumulate and multiply in the sludge treatment facility (Pestana et al. 2016; Zamyadi et al.2016) and if the cyanobacteria in the sludge lyse and release metabolites into the supernatant (Pestana et al. 2016). If cell lysis occurs during sludge treatment, this could result in recycling all metabolites back into the plant. Even though supernatant may represent a low percent of total flow (typically 8–10%) this practice has the potential to severely compromise finished water quality.

Treatment of cyanobacteria-laden sludge has historically gained little attention in the scientific literature. The limited information previously available suggested that cyanobacteria, once incorporated into a floc, rapidly lose viability and release metabolites such as the cyanotoxins and taste and odour compounds MIB and GSM (Drikas et al. 2001). More recent reports (Ho et al. 2012a, 2013; Sun et al. 2012, 2013; Pei et al. 2014; Li et al.2015; Pestana et al.2016) indicate that cyanobacteria in sludge do not necessarily lose viability and lyse simultaneously. In fact, some cells may remain viable for up to 10 days or more in the sludge, thereby providing a prolonged risk to water quality if supernatant is returned, and additional uncertainty regarding withholding periods for facilities treating cyanobacteria-laden sludge.

In a recent investigation, Pestana et al. (2016) found that the risk of recycling sludge supernatant may be higher than can be estimated by the cell and metabolite concentrations entering the treatment plant. The authors studied a range of cultured and environmental cyanobacteria samples and observed that, in the absence of rapid biological biodegradation, the final metabolite concentration in sludge supernatant can exceed the initial mass by a factor of up to five times. The authors attributed this finding to an increased metabolite production in the cyanobacteria captured in the sludge, a proliferation of cyanobacteria in the sludge, or a combination of these factors. This finding potentially has significant implications for the management of sludge both within the treatment plant and in sludge treatment facilities where the supernatant is recycled to the head of the plant or released to the environment. However, the complexity and interrelated nature of the processes that may contribute to the release, production, biodegradation and physical and chemical reduction of metabolites in the sludge and supernatant render the accurate assessment of the risk to water quality an extremely difficult task.

A parameter that has not yet been studied to aid in the understanding of these processes is intracellular organic material (IOM) measured using the bulk parameter of dissolved organic carbon (DOC). When cyanobacterial cells are damaged, the cell wall integrity becomes compromised and IOM is released into the extra-cellular matrix (Jones & Orr 1994; Coral et

al.2013; Korak et al. 2015). This IOM consists of a range of compounds such as proteins, lipids and polysaccharides (Henderson et al. 2008; Zhang et al. 2014; Korak et al. 2015) as well as the secondary metabolites such as toxins and taste and odour compounds. There is also organic matter associated with the extracellular matrix and the cell membrane itself, which can also vary significantly between species and the total organic material is sometimes referred to as algogenic organic matter (AOM) (Henderson et al. 2008; Lei et al. 2014). IOM is generally considered to contribute the majority of the total organic matter associated with cyanobacteria (Lei et al. 2014).

The amount and character of organic matter associated with cyanobacteria has been studied extensively (for example Henderson et al. 2008; Lei et al. 2012, 2014; Zhang et al. 2014; Korak et al. 2015), but not in relation to release during sludge treatment processes. Although IOM is known to be biodegradable due to the high content of low molecular weight proteins and lipids (Nguyen et al. 2005), if the rate of degradation is significantly slower than the rate of release, IOM may be a valuable parameter to aid in the understanding of the behaviour of cyanobacteria in the sludge blanket.

The present state of knowledge suggests that the inherent risk in supernatant recycling may be greater than can be determined by a mass balance of metabolites entering the plant. The aim of this study was to further clarify the potential for cyanobacteria to survive and produce metabolites in sludge treatment facilities using a range of cyanobacteria in waters of different quality. The outcomes will lead to a greater understanding of the complex processes occurring in cyanobacterial sludge treatment, and the ability to more accurately assess risk to drinking water quality associated with common supernatant recycling practices.

MATERIALS AND METHODS

Cyanobacterial cultures

Microcystis aeruginosa (ref strain MIC338), Cylindrospermopsis raciborskii (ref strain CYP011K), and Dolichospermum circinale (formerly known as Anabaena circinalis, ref strain ANA188B) were sourced from the Australian Water Quality Centre culture collection and cultured in ASM-1 (Gorham et al. 1994) at 20 W C under a 12 h/12h light/dark cycle at an intensity of 70 µmol photons m^2 s^{-1}. These strains produce microcystin LR and LA (MC-LR and MC-LA), cylindrospermopsin (CYN) and GSM respectively. The cells were harvested at the end of the exponential phase to maximise the cell number and viability.

Cell enumeration was performed with a light micro- scope (Nikon 50i, Japan) at 200 times magnification using a Sedgewick–Rafter counting chamber. Samples were preserved with Lugol's iodine.

Waters

Raw water was obtained from the inlet of two water treatment plants (WTPs) and stored at 4 W C. Raw water A (RWA) is a reservoir water with generally stable quality. The average turbidity of RWA over the past 10 years was 2.5 NTU (0.6–17.0 NTU), and the DOC is generally high (average 12.2 mg L^{-1}, 8.7–18.5 mg L^{-1}). Raw water B (RWB) is a river water with a very large catchment area (>1,000,000 km^2) and consequently experiences variable water quality. The average turbidity of RWB over the past 10 years was 45 NTU (2–270 NTU), and the DOC is also variable (2–17.6 mg L^{-1}), with an average of 5.9 mg L^{-1}.

Determination of DOC cell quotas

Triplicate raw water samples were inoculated with cyanobacterial species (M. aeruginosa, C. raciborskii, or D. circinale). The inoculated sets of samples were divided into a test group and a control group. Samples from the control group were syringe filtered (<0.45 μm) and the DOC content was determined. Samples from the test group were subjected to three freeze-thaw cycles, followed by syringe filtration (<0.45 μm), and the same analysis as the control group. Calibration graphs were constructed by plotting the DOC increase on cell lysis (test–control) as a function of the cyanobacterial cell concentration.

Simulated sludge lagoon treatment

Two 5 L beakers of raw water containing a nominal concentration of 3×10^5 cells mL^{-1} of cyanobacterial culture (M. aeruginosa, C. raciborskii, or D. circinale) were coagulated using aluminium sulphate (alum) as $Al_2(SO_4)_3 \cdot 18H_2O$ (80 mg L^{-1}, pH 6.3) by flash mixing for 1 minute (480 G) with an Ultra Torque BDC 1850 overhead stirrer fitted with a 75 × 25 mm straight blade impeller (Caframo, Canada) in custom-made 5 L Perspex gator jars, followed by slow mixing (18 G) for 15 minutes. Samples were then allowed to settle overnight. On the following day, the supernatant was decanted, both sludge samples were consolidated in one beaker, and approximately 4.5 L of the respective WTP's sludge lagoon supernatant (sampled from the full scale plant and stored at 4 W C until used) was added in order to bring the total volume to 5 L. Samples from the supernatant were collected at intervals for analysis (metabolite concentration, DOC, UV_{254}). This procedure was also undertaken in the absence of cells to serve as a control.

Sample preparation and metabolite analysis

MC and CYN samples were pre-concentrated from water samples by solid phase extraction according to the methods described in Nicholson et al. (1994) and Metcalf et al. (2002) respectively. All concentrated MC and CYN samples were analysed on an Agilent Technologies 1100 series high performance liquid chromatography system consisting of a quaternary pump (G1311A), degasser (G1379A), auto sampler (G1313A), column compartment (G1316A) and photodiode array detector (G1315B) using a method adapted from Ho et al. (2006) for MCs and Ho et al. (2011) for CYN. The limit of quantification (LOQ) for both methods was 0.05 μg L^{-1}.

GSM samples were concentrated with a solid phase micro extraction syringe fibre (Supelco, Australia) and analysed on a 7890A Gas Chromatograph System with a 5975C VL Series Mass Selective Detector (Agilent Technologies, Australia) against quantified deuterated internal standards (Ultrafine Chemicals, UK) according to a method developed by Graham & Hayes (1998). The LOQ for this method was 4 ng L^{-1}.

DOC analysis

For the determination of DOC, samples were filtered through 0.45 μm pre-rinsed membranes and analysed using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA).

RESULTS AND DISCUSSION

A series of experiments was designed to simulate the release of metabolites from cyanobacteria-laden sludge under a range of conditions. Raw water was obtained from the

inlet of two WTPs. RWA is a reservoir water with generally stable quality; RWB is a river water displaying variable water quality. Three cultured cyanobacteria were studied: Microcystis aeruginosa, producing MC-LR, MC-LA; Cylindrospermopsis raciborskii, producing CYN; and Dolichospermum circinale (previously Anabaena circinalis), producing GSM.
The initial conditions for each experiment, the percent cyanobacteria removal and the water quality parameters at the commencement of the simulated lagoon treatment experiment (day 0) are given in the Supplementary information (available with the online version of this paper).

Organic carbon cell quota

In order to quantify the potential release of IOM by the three cyanobacterial species used in these experiments (M. aeruginosa, D. circinale, and C. raciborskii), samples at a range of cell concentrations were lysed and the release of DOC was measured. A calibration curve was constructed for each species where the increase in DOC on cell lysis, relative to a control that was not lysed, was plotted against the cell concentration. A linear relationship was found for each species; the fitting parameters are given in Table 1. An estimate of the DOC per cell (or cell quota) and the estimated biovolume (VDEPI) is also given in the table. Biovolume is the volume of each cell calculated according to the morphology and size of the cell. In the calculation of the cell quota, the mass of organic carbon per cell is given in Table 1. This is simply the total DOC released from the cells, taking into account the appropriate controls, divided by the number of cells. As some cells are larger, and therefore have a larger volume, it is also appropriate to compare the cells in terms of organic carbon per unit biovolume, also given in Table 1. From these results, it appears that the C. raciborskii culture has an IOM quota per unit biovolume, as measured by DOC, an order of magnitude higher than the other two species. Other characterisation techniques were applied to IOM from the three cultures (UV absorbance, molecular weight distributions using fluorescence and UV detection) and no significant differences were observed (data not shown). It has been reported that IOM quota varies by orders of magnitude between species, and the quota and character are also dependent on the growth phase of the culture, growth conditions such as light, temperature, nutrients etc. (Henderson et al. 2008; Leloup et al. 2013; Korak et al. 2015). Limited data exist in the literature regarding cyanobacteria DOC quota; Table 2 lists available literature values for M. aeruginosa compared with the values found in this study. These results indicate that cell quotas are variable and are dependent on strain as well as the range of environmental conditions. The efficiency of lysis and extraction methods may also contribute to the errors involved in this analysis.

Table 1 - Fitting parameters of the relationship between DOC increase and cell concentration (n = 6)

Species	R^2	Gradient (mg DOC cell ^{1})	Biovolume (VDEPI) (μm^3)	DOC μm ^{3}
M. aeruginosa	0.96	7.7×10^{10}	87	9×10^{12}
D. circinale	0.92	1.1×10^{9}	250	4×10^{12}
C. raciborskii	1.0	1.6×10^{9}	42	4×10^{11}

Table 2 - Literature values for IOM quotas, M. aeruginosa

Cell quota (mg DOC cell 1)	Publication
7.7×10^{-10}	This paper
5×10^{-10}	Lei et al. (2012)
6.4×10^{-9}	Coral et al. (2013)
9.5×10^{-13}	Henderson et al. (2008)
1.4×10^{-8}	Shen et al. (2014)

For all experiments reported here, the cyanobacteria cultures were grown in the same conditions and harvested at the same point in the growth curve using the same methods to minimise as far as possible, variations caused by these factors.

Release of DOC from sludge during simulated sludge treatment

RWA and RWB were inoculated with cyanobacterial cells and coagulated using alum. Control experiments were also undertaken in the absence of cyanobacteria. After settling for 1 day, the supernatant was replaced with sludge lagoon supernatant from the respective WTP, this is designated as *Time = 0 (T = 0)* in the figures and discussion of results. The increase in DOC in the supernatant was monitored from *T = 0* over a period of between 15 and 22 days. This procedure was used to simulate the removal of sludge from the clarifiers and subsequent lagoon storage.

For the control jars and those containing cyanobacteria, an increase in the supernatant DOC concentration was observed over the period of the experiment, indicating that organic matter was also released from sludge in the absence of cyanobacteria. The increase in DOC attributable to cell lysis and release of organic matter from the cyanobacterial matrix within the sludge (observed concentration, C_o) was determined by subtracting the increase in DOC in the control from the increase in DOC in the test jar. This was considered an estimate of the organic material contributed to the supernatant from the cyanobacteria over time. The total IOM (measured as DOC) that could be expected from the cyanobacteria, assuming all cells were lysed completely (predicted concentration, C_p), was calculated using the number of cells captured in the sludge (Supplementary information Table 1, available with the online version of this paper) multiplied by the IOM quotas per cell shown in Table 1. The ratio of C_o to the predicted maximum (C_p) was then plotted as a function of time in days. In Figure 1 this is shown as C_o/C_p (observed concentration increase due to cyanobacteria divided by predicted concentration) vs time.

The release in general follows a linear trend, and in most experiments the C_o/C_p was still increasing at the termination of the experiment. In addition, most of the values of C_o/C_p at the termination of the experiments exceeded 1, i.e. the observed increase in DOC was higher than that expected from the initial cell numbers present in the closed systems. In particular, M. aeruginosa in RWA, experiment 1, appeared to have a very large increase in DOC within a week, with the C_o/C_p at 14 days >5. This large increase in DOC was confirmed by the increase in UV absorbance at 254 nm, which doubled from 0.12 at day 0 to 0.24 at day 16 (data not shown). However, in the other experiments the C_o/C_p at termination of the experiment was lower, between 1 and 3.8.

Although there are uncertainties inherent in these estimations, the high values of C_o/C_p may suggest that during the period of the experiment:

- cells produced higher levels of IOM or AOM due to stress, which was released over time, and/or
- the cells were multiplying in the sludge simultaneously with other processes such as loss of viability and lysis.

A variation in AOM with different stages in the growth phase has been reported previously (Pivokonsky et al. 2006; Henderson et al.2008); however, the possibility of increased production and release due to stress has not been reported. Although the multiplication of cells is unexpected due to the loss of mobility and light limitation in the sludge, it is considered possible as it has previously been reported, under similar experimental conditions, that up to 80% of cyanobacteria in sludge maintained viability for prolonged periods (Ho et al. 2012a, 2013), and that they may continue to produce metabolites over that period (Pestana et al. 2016). Results of investigations at the full scale also suggest that cyanobacteria are robust microorganisms that can accumulate and continue to be viable in clarification basins and in the sludge blanket (Zamyadi et al. 2012, 2013). The difference in the release of DOC between experiments 1 and 2 in RWA is significant, and may indicate a difference in the health of the M. aeruginosa culture when harvested (Figure 1(a)). The M. aeruginosa curve in Figure 1(b) displays similar behavior to experiment 2, Figure 1(a)) where the Co/Cp DOC remained below 1 for the extent of the experiment.

Release of secondary metabolites during simulated sludge treatment

Figures 2 and 3 show the variation in toxin concentration over time in the supernatant, plotted as measured concentration divided by predicted maximum concentration (Co/Cp). The predicted concentration was determined from the initial conditions (Supplementary information Table 1). Under all conditions where toxins were monitored, the toxin concentrations increased then decreased, indicating lysis of the cyanobacteria in the sludge and release of the metabolites followed by biological degradation. In both waters, the release of CYN occurred after a lag of 2 or 3 days, consistent with the DOC release curves (Figure 1), and reached a maximum around 14 days (Figure 3). In contrast, the MC toxins appeared to be both released and degraded more rapidly, with the toxin concentration below detection by 15 days. The only previous studies on the release and degradation of toxins from coagulated cyanobacteria also showed no consistency in terms of rates of release and degradation and observed lag phases (Ho et al. 2012a, 2013; Maghsoudi et al. 2015), indicating a strong dependence on experimental and environmental conditions.
The results in the current study suggest that MC degrading organisms occur in RWA, and CYN degrading organisms occur in both waters. Degradation of MC occurred more rapidly in RWA than the degradation of CYN, as can be seen by the number of days for the concentration to decrease to below detection. The lower rate of biodegradation of CYN than MC is in agreement with the work of Ho et al. (2012b), who compared the rates of degradation of both toxins in a range of raw water sources. In addition, an extended lag phase prior to CYN degradation has been reported previously and has been attributed to the fact that the indigenous microorganisms have not been acclimated to the presence of CYN (Smith et al. 2008). Neither of the raw waters used in this study had previously experienced a toxic bloom containing CYN. The concentration of dissolved MC-LR in RWA experiment 2 reached a maximum concentration close to the expected maximum concentration within 2–3 days, prior to degradation occurring (Figure 2). This is in agreement with the DOC results for this experiment (Figure 1(a)) where C_o/C_p reached 0.75 within 2 days, then remained relatively stable around 1.

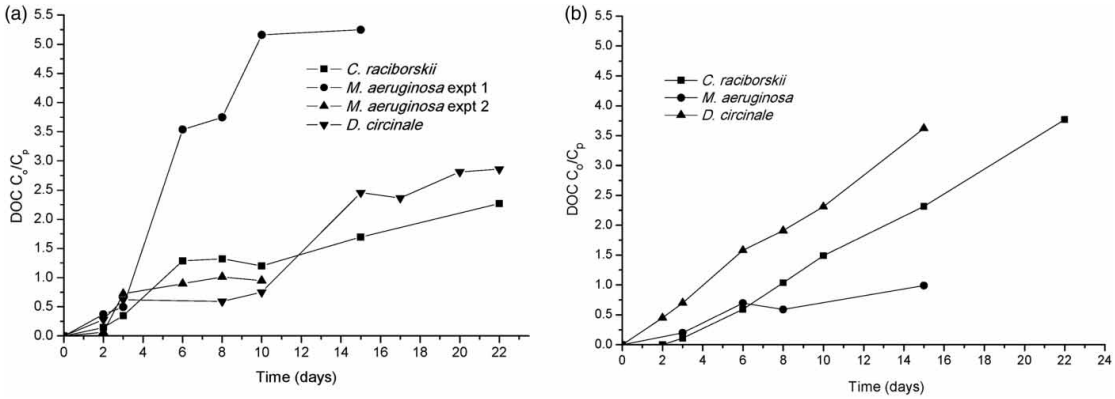

Figure 1 **-** Increase in DOC in sludge supernatant over time, corrected for the control, represented as the ratio of observed to predicted values (Co/Cp). RWA (a), RWB (b).

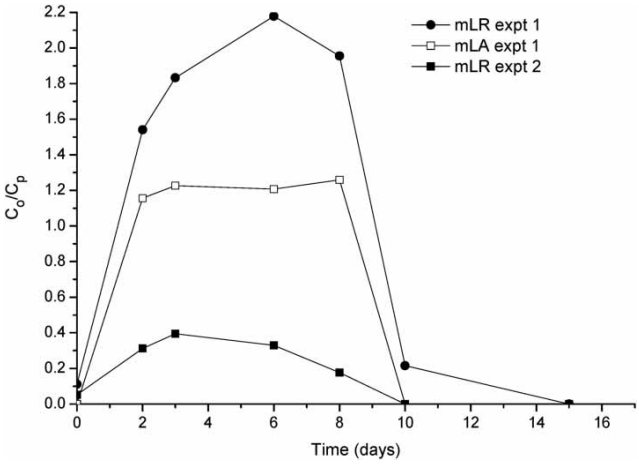

Figure 2 - Increase in MC concentration in sludge supernatant over time, represented as the ratio of observed to predicted values (C_o/C_p). RWA.

For the other toxin experiments reported here, the maximum concentrations were higher than expected considering the number of cells initially coagulated ($C_o/C_p > 1$). In the case of CYN and MC-LR, significantly higher toxin than predicted was detected in the extra-cellular matrix. In these cases the C_o/C_p for DOC was also above 1.

Toxin production by cyanobacteria has been studied extensively and has been shown to be influenced by a range of conditions such as growth phase, light intensity, nutrient levels and temperature, although the findings are often contradictory and appear to be dependent on issues such as species, strains, cell concentration (i.e. proximity) and experimental methods (for example using cell number or dry weight of cyanobacteria) (Chorus & Bar- tram 2002; Falconer 2005; Granéli et al.2006; Pimentel & Giani 2014; Yang et al. 2015). As the measurement of metabolite concentration in the supernatant is an indirect measure of cell damage and lysis within the sludge blanket, it is unclear whether the higher than expected toxin release was due to increased production per cell, cell multiplication in the sludge, or a combination of both processes. However, it is clear from these findings, supporting those of Pestana et al. (2016), that an increase in metabolite production within sludge treatment facilities may occur with a range of cyanobacteria species, metabolites and water quality.

In RWA, the concentration of GSM in the supernatant after coagulation of D. circinale was below detection for the extent of the experiment, although the C_p of GSM was 1,500 ng L^{-1}.

Figure 4 shows the concentration of extracellular GSM in RWB, the dotted line represents C_p in this experiment. C_o/C_p was not plotted for these data as it was very low (maximum 0.05) compared with the other metabolites and DOC. This result is in contrast to the DOC C_o/C_p for D. circinale coagulated in both waters, which reached 2.5 and 3.5 after 14 days in RWA and RWB respectively. The fact that little or no extra-cellular GSM was detected in samples in either water compared with the predicted value is most likely due to the fact that GSM readily volatilises, adsorbs to natural organic material (NOM), and rapidly biodegrades in most water sources (Watson et al. 2000; Ho et al.2007). In these experiments, it is likely that the rate of biodegradation of GSM is similar to, or greater than, the rate of release from cells and consequently little or no GSM is detected in the dissolved form. In addition, there is regularly GSM present in both source waters, and high rates of degradation in these waters have been reported previously (Ho et al. 2007, 2012a; Hoefel et al. 2009; McDowall et al. 2009).

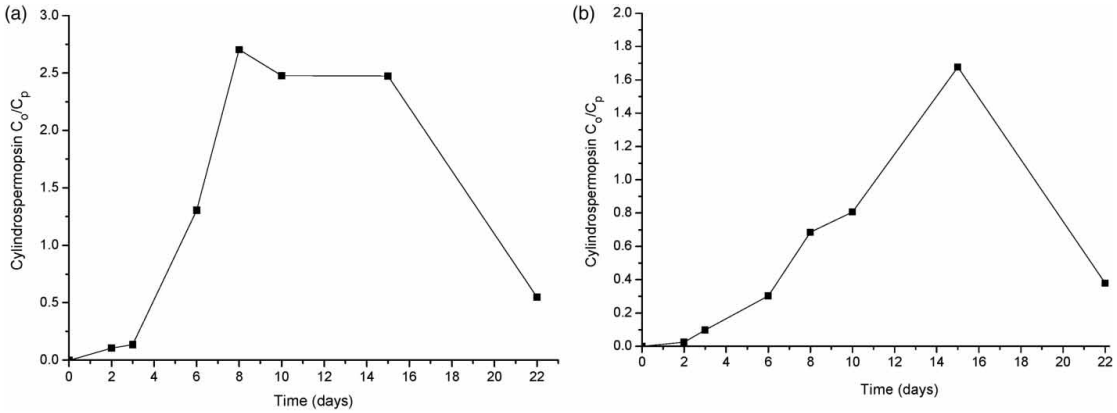

Figure 3 - Increase in CYN concentration in sludge supernatant over time, represented as the ratio of observed to predicted values (C_o/C_p). RWA (a), RWB (b).

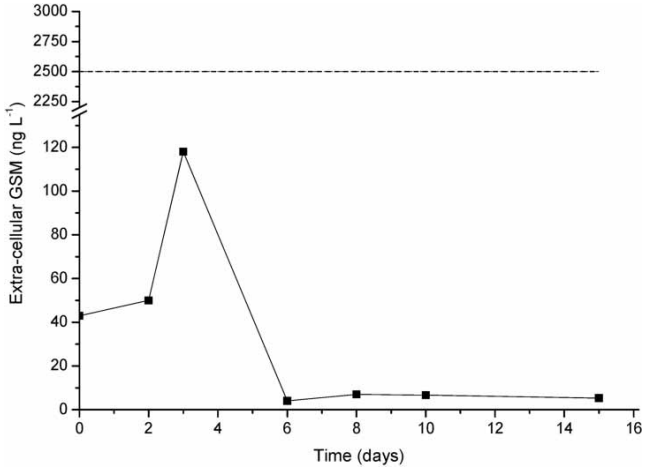

Figure 4 - Increase in extracellular GSM concentration in sludge supernatant over time, dotted line represents predicted maximum concentration, RWB.

Operational implications and risk assessment

Table 3 summarises some basic parameters estimated from the limited data available on release and degradation of a range of metabolites in the presence of water treatment sludge. The data are compared in terms of estimated lag phase prior to commencement of metabolite release, time in days taken for release of half the maximum predicted concentration ($t_{0.5R}$) and time taken for degradation to half the observed maximum concentration ($t_{0.5D}$).
The order of rate of damage and metabolite release based on the results presented in Table 3 is: *D. circinale* > *M. aeruginosa (Microcystis sp.)* > *C. raci- borskii* > *Pseudanabaena*
However, it should be noted that these results may be influenced by the effect of simultaneous release and degradation of the associated metabolites. Based on the IOM results discussed above, the order of susceptibility can be compared based on the rate constants of the apparent zero-order rate of release of organic material. The order based on these rate constants (taking into account the variations shown in Figure 1) is given below:
D. circinale ≥ *M. aeruginosa* ≥ *C. raciborskii*
The order of biodegradability of related metabolites in a range of systems is:
GEO > MIB > MC-LR (LA) > CYN > STX

This latter order is supported by papers related to the degradation of metabolites in the environment, in the presence of laboratory bacteria cultures and in biological filters (for example Ho et al. 2007, 2012b, 2012c; Kayal et al. 2008; Hoefel et al. 2009).
In addition, the results in this paper and Pestana et al. (2016) suggest that the less biodegradable metabolites (MIB, MC-LR, MC-LA, CYN and STX) pose the additional risk of increasing in concentration in the sludge from 1.5 up to 5-fold. Overall, the results indicate that, once captured in sludge, cyanobacteria may continue to produce and release metabolites for more than a week in a closed batch system. In a dynamic system such as a lagoon where sludge is constantly replenished, the risk to water quality will be compounded and ongoing for the period of the bloom and several weeks beyond that time. Using the procedures outlined by Bartram et al. (2009), taking into account the likelihood of impact on quality and the consequences to aesthetics and public health, an assessment of the risk of supernatant recycling is shown in Table 4.

Table 3
Comparison of parameters associated with release and degradation of metabolites in sludge.

Cyanobacteria	Metabolite	Lag (days)	$t_{0.5R}$ (days)	$T_{0.5D}$[a] (days)	Source
M. aeruginosa	MC-LR	<2	<2	<1-3	This paper
M. aeruginosa	MC-LA	<2	<2	<1	
D. circinale	GSM	2	NA	<3	
C. raciborskii	CYN	2-3	3-8	4	
C. raciborskii	CYN	<1-2	3-4	6[b]	Ho et al. (2012a), (2013) and Maghsoudi et al. (2015)
M. aeruginosa	MC	<1-13	1-6	1-4	Ma et al. (2012), Sun et al. (2012), Pei et al. (2014), Maghsoudi et al. (2015) and Pestana et al. (2016)
D. circinale	GSM	<1	<1-1	2-5	Ho et al. (2013) and Pestana et al. (2016)
D. circinale	STX	<1	2	NA	Ho et al. (2012a) and Pestana et al. (2016)
Pseudanabaena	MIB	<1-3	4-12	2-3	Ho et al. (2013) and Pestana et al. (2016)

NA – not applicable. [a]Where degradation was observed. [b]One literature source only.

Table 4 summarises the information brought together in this paper and the known efficiencies of various treatment processes (see summaries in Newcombe et al. (2010) and WHO (2015)) to produce a qualitative estimate of the residual risk to aid in the identification of high risk

practices associated with sludge supernatant recycling in the presence of a cyanobacteria. The assumption is that all metabolites in the supernatant are extracellular.
The final assessment of the risk to finished water quality of sludge supernatant recycling in the presence of cyano- bacteria is dependent on the individual and cumulative removals of dissolved cyanobacterial metabolites achieved by each treatment barrier in the treatment plant.

Table 4 - Summary of risks associated with supernatant recycling based on different cyanobacteria and water treatment process efficiencies: L-low; M-medium; H-high; VH-very high

Cyanobacteria	*Pseudanabaena*	*D. circinale*		*M. sp.*	*C.raciborskii*
Metabolite	MIB	GEO	STX	MCs	CYN
Risk from supernatant recycling	H	M	VH	VH	VH
Treatment barrier	Residual risk associated with individual treatment barriers				
Powdered activated carbon (PAC)[a]	M	L	M	M	M
Coagulation	H	M	VH	VH	VH
Ozone	M	M/L	L	L	L
Granular activated carbon (GAC) (physical removal)[b]	M	M/L	M/H	M/H	M/H
GAC (biological removal)[c]	L	L	VH	M	M/H
Chlorine CT > 50 mg L^{1} min^{1}	H	M	L	L	L

[a]Good quality PAC, 20 mg/L, 30 min contact time.
[b]Dependent on the GAC remaining adsorption capacity.
[c]Dependent on the presence of degrading bacteria in the biofilm.

SUMMARY AND CONCLUSIONS

The assessment of risk and subsequent operational decision-making regarding the occurrence and extent of sludge supernatant recycling during a cyanobacteria challenge will ideally be based on specific knowledge of the processes (physical, chemical and biological) taking place in the treatment facility. If such detailed knowledge is available, water suppliers can determine the appropriate time to reduce or terminate recycling, for example, due to unacceptable risk to the finished water quality. These decisions are important operationally, as the termination or reduction of recycling can have implications regarding alternative disposal of supernatant of compromised quality (e.g. disposal to sewer or to the environment).
This research has demonstrated that recycling sludge supernatant during a cyanobacteria bloom poses a higher risk to finished water quality than can be estimated from a mass balance using inlet concentrations. The release of algal organic material and metabolites from coagulated cyanobacteria demonstrated that the cells remain in the sludge producing and releasing intracellular material for prolonged periods. In a dynamic system such as a sludge lagoon, this will result in compromised water inlet water quality for the length of the cyanobacterial challenge, and potentially substantially longer if supernatant recycling is continued.
Due to its rapid release and degradation in most environments, GSM exhibits the lowest risk to water quality. In contrast, MIB, MCs, saxitoxins and CYNs pose a significant risk to water quality and safety if the practice of supernatant recycling is continued. The residual risk to water quality can be minimised by applying robust, effective multiple treatment process barriers that have been monitored and verified for dissolved cyanobacterial metabolite removal.

Knowledge of the potential risks of supernatant recycling, and the effectiveness of the treatment barriers that are in place to mitigate those risks, is essential for the supply of safe, clean drinking water during a cyanobacterial challenge.

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SUPPLEMENTARY INFORMATION

Table 1 | Water quality parameters. Initial conditions represent the parameters after adding the cyanobacteria culture to the water sample, prior to coagulation. After coagulation and settling for 1 day, the supernatant was replaced with sludge lagoon supernatant from the respective water treatment plant, this is designated as Day 0. NM – not measured; BD – below detection; intra – intracellular; extra – extracellular

***D. circinale* RWA**

Parameter	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA ($L mg^{-1} m^{-1}$)	Cell count (cell mL^{-1}) [percent cell removal]	GEO_{intra} (ng L^{-1})	GEO_{extra} (ng L^{-1})
Initial	9.1	0.30	3.9	350,000	740	BD
Day 0	5.6	0.10	1.9	2162[99]	BD	BD

***M. aeruginosa* RWA**

Parameter	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA ($L mg^{-1} m^{-1}$)	Cells (cell mL^{-1})	$MCLR_{intra}$ (µg L^{-1})	$MCLR_{extra}$ (µg L^{-1})	$MCLA_{intra}$ (µg L^{-1})	$MCLA_{extra}$ (µg L^{-1})
Expt 1 Initial	10.1	0.35	3.4	310,000	5.0	2.9	1.7	BD
Expt 1 Day 0	5.2	0.12	1.9	2760[99]	BD	4.0	BD	BD
Expt 2 Initial	11.5	0.34	2.9	233000	4.7	2.0	BD	BD
Expt 2 Day 0	6.5	0.13	2.0	4300[98]	0.5	2.5	BD	BD

***C. raciborskii* RWA**

Parameter	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA ($L mg^{-1} m^{-1}$)	Cells (cell mL^{-1})	CYN_{intra} (µg L^{-1})	CYN_{extra} (µg L^{-1})
Initial	10	0.33	3.4	311,200	2.5	0.7
Day 0	6.0	0.12	2.0	7080[98]	1.0	0.8

***D. circinale* RWB**

Parameter	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA (L mg^{-1} m^{-1})	Cells (cell mL^{-1})	GEO_{intra} (ng L^{-1})	GEO_{extra} (ng L^{-1})
Initial	8.5	0.19	2.31	308,750	1246	4
Day 0	4.3	0.074	1.71	143750[53]	844	43

***M. aeruginosa* RWB**

Parameter	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA (Lmg^{-1} m^{-1})	Cells (cell mL^{-1})	MC-LR	MC-LA
Initial	8.0	0.203	2.5	NM	NM	NM
Day 0	4.1	0.05	1.2	NM	NM	NM

***C. raciborskii* RWB**

Parameter	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA (L mg^{-1} m^{-1})	Cells (cell mL^{-1})	CYN_{intra} (µg L^{-1})	CYN_{extra} (µg L^{-1})
Initial	8.63	0.24	2.8	311,000	2.7	0.8
Day 0	4.9	.08	1.7	4140[99]	0.3	0.9