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# Treatment challenge of a cyanobacterium Romeria elegans bloom in a South Australian wastewater treatment plant – a case study.

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**Abstract**
A bloom of the non-toxic cyanobacterium Romeria elegans in waste stabilisation ponds (WSPs) within Angaston waste water treatment plant (WWTP) has posed an unprecedented treatment challenge for the local water utility. The water from the WSPs is chlorinated for safety prior to reuse on nearby farmland. Cyanobacteria concentrations of approximately 1.2 × 106 cells mL−1 increased the chlorine demand dramatically. Operators continuously increased the disinfectant dose up to 50 mg L−1 to achieve operational guideline values for combined chlorine (0.5-1.0 mg L−1) prior to reuse. Despite this, attempts to achieve targeted combined chlorine residual (CCR) failed. In this study, samples from the waste stabilisation pond at Angaston WWTP were chlorinated over a range of doses. Combined chlorine, disinfection by-product formation, cyanobacteria cell concentration, Escherichia coli inactivation, as well as dissolved organic carbon and free ammonia were monitored. This study shows that, in the occurrence of cyanobacterial blooms, CCR does not directly suggest pathogen removal efficiency and is therefore not an ideal parameter to evaluate the effectiveness of disinfection process in WWTP. Instead, E. coli removal is a more direct and practical parameter for the determination of the efficiency of the disinfection process.

**Keywords**
Chlorination; Wastewater; Cyanobacteria; Romeria elegans; Water reuse

## Introduction

South Australia wastewater treatment plants (WWTPs) often utilise waste stabilisation ponds (WSPs) as tertiary treatment. Consecutive WSPs are employed to achieve biological degradation of the wastewater constituents. This is usually followed by a disinfection step employing chlorine dosing to remove potentially hazardous coliform bacteria, for example, *Escherichia coli*. The normal biota of WSPs consists of green algae, cyanobacteria, and other degrading organisms [1,2]. Due to their nature, WSPs are high in nutrients, particularly nitrogen and phosphorus, and can provide ideal conditions for the occurrence and proliferation of cyanobacteria.

Cyanobacteria and their metabolites can pose a hazard to human health and safety as well as have a negative commercial impact for water utilities. Their tendency to appear in mass occurrences, or blooms, makes the treatment of the affected wastewater particularly challenging. One reason for this is the large amount of treatment chemicals required to mitigate the sudden increase of biomass that occurs during bloom periods. In addition, many of the secondary metabolites produced by cyanobacteria can be hazardous to human health and safety. In particular, one group of metabolites, the cyanotoxins, have been shown to cause detrimental health effects in humans and livestock alike [3,4].

In this case study, we report the appearance of a non-toxic cyanobacterium bloom which presented a treatment challenge to the local water utility. A persistent *Romeria elegans* bloom that occurred during the winter season in the WSPs at Angaston WWTP (South Australia, Australia) caused an unprecedented disinfectant demand leading to a series of problems that the local water utility struggled to resolve.

The local water utility's treatment target for disinfection at the Angaston WWTP is to achieve a combined chlorine residual (CCR) of 0.5–1mg$L^{-1}$. Furthermore, the South Australia Department of Health has set incident level concentrations for coliform bacteria (as *E. coli* equivalent) of 2000 organisms per 100 mL for effluent (i.e. discharge into the environment) and 10,000 organisms per 100 mL for re-use (e.g. irrigation) [5]. The chlorine dose is determined by the measured residual at the discharge point and is adjusted according to demand. During the *R. elegans* bloom, operators were unable to detect any CCR even at the maximum chlorine dose that the plant could provide (50 mg $L^{-1}$). This high chlorine dose caused additional operational and water quality problems when the dosing lines (chlorine gas inlet) froze due to high amounts of chlorine gas passing through them and the dropping of effluent pH to below 4.

The main aim of this study was to determine whether the operational CCR target was necessary to achieve adequate disinfection of the effluent, as determined by *E. coli* deactivation. For this, the bloom of *R. elegans* from Angaston WWTP was studied and different chlorine doses were applied during simulated disinfection processes to evaluate several key parameters: deactivation of *R. elegans* and *E. coli*, CCR, formation of potentially harmful disinfection by-products (DBPs) and pH of effluent. This allowed the determination of a chlorine dose that achieved acceptable disinfection (determined by *E. coli* levels) while at the same time releasing effluent with an acceptable pH (≥ 6.0).

## Materials and methods

### Angaston WWTP

Angaston WWTP (34°29′23′′S, 139°01′38′′E) is located approximately 90 km north-east of Adelaide on the outskirts of the town of Angaston in South Australia, Australia (Figure 1). The plant was designed to operate at 0.42 megalitres per day on average serving a population of approximately 2000 people. Its treatment process consists of anaerobic digestion followed by WSP treatment in three successive lagoons (average detention time ~16 d). The treated waste water is then disinfected by chlorine dosing and either discharged into a nearby creek or re-used for irrigation of the surrounding vineyards.

### Simulated wastewater disinfection

Wastewater samples from lagoon 3 were chlorinated in triplicate at a range of doses (Table 1), and aliquot samples were taken at three sampling time periods: time 0 (prior to dosing), 5, and 30 min for each dose. These time points were selected to simulate the actual contact time applied for re-use (irrigation) water (5 min) and for discharged water into Angaston Creek (30 min).

### Analysis of WSP water

A range of analytical tests were performed for each sample collected, as described in Table 1 with the addition of temperature and pH monitoring. A chlorine stock solution was prepared by bubbling chlorine gas through ultrapure water to create a saturated stock of 2000–4000 mg $L^{-1}$. The solution concentration and all residual titrations were determined by the DPD-ferrous titrimetric as per Standard Method

4500-Cl (F) [6]. The limits of quantification (LOQ) were between 0.1 and 5.0 mg $L^{-1}$. All chemicals used were of analytical reagent grade. Samples falling outside of the range were diluted with ultrapure water as required.
The ammonia determination was performed with an ion-selective electrode (ISE) according to Standard Method 4500-NH3 (D) [6]. Cell enumeration was performed with a light microscope (50i, Nikon, Japan) at 600× magnification using a Sedgewick-Rafter counting chamber. An aliquot (2 mL) of sample was collected and 25 µL of Lugol's iodine solution was added for sample preservation [7].
Ultraviolet (UV) absorbance at 254 nm (UV254) was measured through a 1-cm quartz cell using an Evolution 60 Spectrophotometer (Thermo Scientific, USA). Dissolved organic carbon (DOC) measurements were con-ducted using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA). Prior to analyses for UV254 and DOC, samples were filtered through 0.45-µm prerinsed membrane filters. Analyses of UV and DOC were performed according to Standard methods [6].
Due to the fact that high doses of chlorine were applied in the simulated disinfection treatments, formation of potentially harmful DBPs was of concern [8,9]. A suite of tests was performed in order to determine the presence and concentration of a wide array of DBPs, which include trihalomethanes (THMs), haloacetic acids (HAAs) and N-nitrosodimethylamine (NDMA). A purge and trap gas chromatographic method according to Standard Method 6232 (C) [6] was used for the analysis of volatile and semi-volatile THMs. Standard Method 6521 (B) [6] was used for the analysis of the nine species of HAAs. NDMA was determined according to a method adapted from [10] using a solid phase extraction method performed to isolate and concentrate the NDMA. An Agilent 6890N GC system with 5973 inert Mass Selective Detector was used. The system was operated in selective ion monitoring mode to increase NDMA sensitivity. A sample volume of 10 µL was injected using Agilent 7683B auto-injector with Helium as the carrier gas (DB-VRX Siloxane column 60 m × 320 µm × 1.8 µm). A calibration curve constructed from NDMA (Accustandard, USA) and NDMA-d6 standards of various concentrations (0, 10, 25, 50, 100, and 300 ng $L^{-1}$) was used to determine the ratio of fragment ions corresponding to a given standard concentration. The LOQ for all DBP measurements was 1 µg $L^{-1}$, with the exception of chloroacetic acid (3 µg $L^{-1}$) and *N*-nitrosodimethylamine (0.3 µg $L^{-1}$).
*E. coli* number was determined by the Colilert-18® method according to [11]. All samples were prepared in triplicate, and results are reported as mean values ± one standard deviation.

## Results and discussion

### Historical data of cyanobacterial bloom at Angaston WWTP

Blooms of toxic cyanobacteria during the summer season have been common and recurrent at Angaston WWTP, especially in lagoon 3 (Figure 2). Historically, the dominant cyanobacterial species in the blooms is a toxigenic *Microcystis sp*. (microcystin-RR producer). However, during a *Microcystis sp.* bloom event in the summer season in February 2014, another cyanobacterium was noticed and was identified as a *Romeria sp*. Due to the non-toxic nature of the *Romeria* genus, identification to species level was not performed at the time. During the winter season of 2014, a bloom was observed at Angaston WWTP, and the dominant species was subsequently identified as *R. elegans*. The identification of this species was performed according to the identification keys described in ref. [12]. The average temperature at the Angaston area during the occurrence of the bloom was recorded to range between 2.4°C (average minimum) and 13.2°C (average maximum) [13]. To the authors' knowledge, this is the first evidence of a bloom of this species in Australia.

Worldwide occurrence of *Romeria sp*. commonly occurs in nutrient-rich and/or heavily polluted water bodies [14–16]. WSPs, by virtue of their functions, thus, provide ideal habitats for this group of cyanobacteria. Several studies have also indicated the ability of *Romeria* group to thrive in climates as varied as central Europe, Alaska, South America, Africa, and Asia [12,17–20]. Similarly, routine monitoring at Angaston WWTP also indicated consistent presence of *R. elegans* over the various seasons in the year of 2014, including its presence during a toxic *Microcystis sp.* bloom over the summer months. A contributing factor to the development of *R. elegans* bloom during the colder season might be the absence of nutrients competition from other commonly occurring cyanobacteria, such as *Micro-cystis sp*. that are normally observed during summer blooms at Angaston WWTP. This argument was supported with historical data for onsite routine monitoring of NH3 (Figure 3), a compound known as primary nitrogen source for non-nitrogen-fixing cyanobacteria [21]. It was apparent from the figure that level of NH3 in the water remained low (< 2.0 mg $L^{-1}$) during the occurrence of two blooms (*Microcystis sp.* and *R. elegans*) in the first three quarters of 2014, indicating its consumption by the cyanobacteria. This was further supported by the subsequent rapid increase of NH3 concentration observed after the collapse of the second bloom in the year (*R. elegans*).

Simulated disinfection of wastewater in the presence of cyanobacteria bloom

Treated water from Angaston WWTP undergoes a disinfection process by chlorination, after which the effluent is either discharged into a local creek (30 min contact time) or re-used for vineyard irrigation (5 min contact time). During the *R. elegans* bloom incident, all treated effluent was re-used for irrigation after a 30-min chlorine contact time. To ensure optimal disinfection, the operational target at Angaston WWTP is a CCR of 0.5–1.0 mg $L^{-1}$, based on the National Guidelines for Water Recycling [22]. The lower limit is to ensure complete removal of pathogenic microorganisms and the upper limit is to ensure a low risk of chemical hazard to commercial crops.

Achieving these values, however, can be difficult particularly in the event of blooms where a massive increase of biomass will consequently increase chemical demand in the treatment process; this was evident during the winter bloom at Angaston WWTP. When the CCR in the effluent decreased below the operational target, the response of the WWTP operators was to increase the chlorine dose with the aim of achieving the targeted levels. This led to additional operational problems, such as increased use of disinfection chemicals (chlorine), which in turn led to freezing of the dosing line, and a low effluent pH.

High chlorine consumption in the wastewater samples can be caused by several parameters, such as NH3 and organic matter. However, as the level of NH3 in the water was historically low during bloom events (0.80 mg $L^{-1}$, Figure 3), it was estimated to have no or little contribution (theoretical HOCl required <2.0 mg $L^{-1}$) to the high chlorine demand. Initial analysis of DOC and UV254 in the wastewater sample from lagoon 3 (pre-chlorination) revealed that organic matter present in the water would provide some chlorine demand (Table 2; DOC = 16 mg $L^{-1}$, UV254 = 0.24 mg $L^{-1}$).

However, based on the normal operation of the plant, the main factor influencing the demand at the plant during the bloom was the cyanobacterial cells (*R. elegans*). Cyanobacteria are known to be vulnerable to chlorination; lysis of cells and release of intracellular organic material occurs rapidly upon contact with chlorine [23–27]. Cyanobacteria are also known to exert a chlorine demand [28] and it is likely that the high numbers of R. elegans cells in Angaston WWTP lagoon water represented a significant contribution to the very high chlorine demand seen at the full scale.

In this study, it was shown that R. elegans cell concentration decreased with increasing chlorine dose with approximately 60% reduction at a dose of 50 mg $L^{-1}$ (Figure 4). However, considering the limitations in cell counting method, the actual loss of R. elegans viability may have been higher than the reduction of cell concentrations observed. In addition, lysis of *R. elegans* cells upon chlorination

appeared to occur rapidly and reached completion within 5 min, indicated by the similar level of reductions between two contact times (5 and 30 min). This is supported by the CCR levels given in Table 2, which shows that the majority of the chlorine dosed had been consumed within 5 min, and only the 30, 40, and 50 mg $L^{-1}$ doses resulted in a measurable CCR. However, these CCR values were very low indicating that even at these high doses most of the reaction had taken place within 5 min.

At a high level of cyanobacteria cell concentration, the release of intracellular organic matter (IOM) that occurs during the cell lysis process can result in a measurable increase of DOC concentration. This was also observed when analysing DOC for Angaston WWTP lagoon water that has been treated to achieve complete cell lysis (microwave treatment). It was noted that the cyanobacteria in the water sample contributed to an increase of approximately 2.2 mg $L^{-1}$ DOC. In addition to DOC, previous work has also shown that the release of IOM causes an increase in the UV absorbance to the surrounding matrix [27]. Therefore, it was of interest in this study to also monitor levels of DOC and UV absorbance prior to and after the simulated chlorination process.

Results in this study revealed no trend in the effect of chlorine dose and contact time on the measured DOC levels (Table 2). However, measurement of UV254 showed an increase in absorbance level with increasing chlorine dose and displayed a linear relationship with the reduction in cell numbers (Figure 5). These results suggest that the chlorine reacted with organic material in the lagoon water and with the organic matter associated with cyanobacteria. The resulting compounds were not detectable as CCR or DBPs, since concentrations of both parameters were relatively low compared to the mass of chlorine consumed in the reaction (Table 3).

Such low level of DBPs formation in chlorinated water with the presence of cyanobacteria was also demonstrated in the work of Zamyadi et al. [26]. In addition, the low pH level of the water caused by the high chlorine dose may also contribute to the low formation of DBPs [29]. This suggests that the chlorination of Angaston WWTP bloom samples produced other DBPs that are not identified in this study.

Although the operational target of a CCR of 0.5–1.0 mg $L^{-1}$ was not achieved under any conditions tested in the laboratory or in the plant, approximately 41% reduction of the indicator organism *E. coli* was evident after 30 min at a chlorine dose of 10 mg $L^{-1}$ (Table 2). Further reductions were seen at 30 and 50 mg $L^{-1}$ chlorine doses. This suggests that the organism was susceptible to disinfection within the first 5 min post-chlorination, and the operational CCR target was not required under these conditions. As the initial cell numbers of E. coli were relatively low, the operational decision was taken to reduce the chlorine dose at the plant to 20 mg $L^{-1}$, resulting in lower chemical costs and fewer operational issues associated with dosing high concentrations of chlorine. It is not possible from these results to determine whether a very short contact time with chlorine or the disinfectant power of unidentified chlorination by-products was responsible for the reduction in viability of the organism. However, the results indicate that a reduction in chlorine concentration under high demand conditions may be advisable to achieve chemical and operational savings. Investigations into the disinfection of wastewater effluent in the presence of cyanobacteria are continuing.

## Conclusion

This study showed that during a cyanobacterial bloom of *R. elegans* the presence of large algal biomass increased the chlorine demand during the disinfection process. Due to the increased chlorine demand, the local water utility struggled to achieve a CCR target and consequently kept increasing the disinfectant dose, which led to an unacceptably low pH in the effluent. It was demonstrated that disinfection by chlorination at a lower dose than was applied at the WWTP achieved adequate disinfection in the absence of a CCR, as indicated by the level of *E. coli* deactivation. The common strategy of increasing the chlorine dose to achieve the target CCR in

this case resulted in higher costs and operational issues. Instead, it is recommended that operators consult the coliform removal data to obtain a direct indication of the disinfection efficiency and base the decision whether or not to increase the disinfectant dose on this data. This study has highlighted the need for a change in operational attitude as well as the need to review operational target parameters. A systematic study in a controlled laboratory environment will increase understanding of the processes taking place in this complex system, and aid in the further development of disinfection guidelines for wastewater in the presence of cyanobacteria.

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**Table 1.** Range of tests performed on samples of the *R. elegans* bloom event at Angaston WWTP.

| Cl dose (mg $L^{-1}$) | CCR | $NH_3$ | Cells | DOC | $UV_{254}$ | THM | HAAs | NDMA | *E.coli* |
|---|---|---|---|---|---|---|---|---|---|
| 0 | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | ✓ |
| 5 | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | × |
| 10 | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | ✓ |
| 15 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × |
| 20 | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | × |
| 30 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 40 | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | × |
| 50 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

Note: CCR: combined chlorine residual; NH3: free ammonia; cells: cell concentration; DOC: dissolved organic carbon; UV254: UV absorbance at 254 nm; THM: trihalomethanes; HAAs: haloacetic acids; NDMA: N-nitrosodimethylamine; E. coli: Escherichia coli concentration.

**Table 2.** Water quality parameters of Angaston WWTP bloom sample pre- and post-chlorination at a range of chlorine doses with 5 and 30 min contact time.

| | | Concentration of components | | | | | |
|---|---|---|---|---|---|---|---|
| Contact time (min) | Chlorine dose (mg $L^{-1}$) | *R. elegans* ($10^6$ cells $mL^{-1}$) | CCR (mg $L^{-1}$) | DOC (mg $L^{-1}$) | $UV_{254}$ | pH | *E. coli* (cells 100 $mL^{-1}$) |
| 0 | 0 | 1.19 ± 0.1 | - | 16.3 ± 0.4 | 0.24 ± 0.0 | 7.6 ± 0.3 | 1600 ± 0 |
| 5 | 5 | 1.09 ± 0.1 | <0.1 | 16.2 ± 0.4 | 0.28 ± 0.0 | - | - |
| | 10 | 1.09 ± 0.0 | <0.1 | 11.9 ± 1.7 | 0.37 ± 0.0 | - | - |
| | 15 | 0.74 ± 0.0 | <0.1 | - | 0.46 ± 0.0 | - | - |
| | 20 | 1.06 ± 0.1 | <0.1 | 11.9 ± 3.5 | 0.36 ± 0.0 | - | - |
| | 30 | 0.77 ± 0.2 | 0.1 ± 0.0 | - | 0.48 ± 0.0 | - | - |
| | 40 | 0.85 ± 0.0 | 0.2 ± 0.0 | 18.6 ± 0.4 | 0.56 ± 0.1 | - | - |
| | 50 | 0.53 ± 0.0 | 0.2 ± 0.0 | 12.1 ± 2.8 | 0.67 ± 0.1 | - | - |
| 30 | 5 | 1.03 ± 0.1 | <0.1 | 14.0 ± 0.3 | 0.40 ± 0.1 | 7.3 ± 0.1 | - |
| | 10 | 0.92 ± 0.2 | <0.1 | 14.1 ± 1.7 | 0.36 ± 0.0 | 6.9 ± 0.1 | 950 ± 42 |
| | 15 | 0.86 ± 0.0 | <0.1 | 14.2 ± 1.1 | 0.31 ± 0.0 | 6.7 ± 0.1 | - |
| | 20 | 1.10 ± 0.1 | <0.1 | 13.4 ± 0.4 | 0.34 ± 0.0 | 6.6 ± 0.1 | - |
| | 30 | 0.69 ± 0.1 | 0.1 ± 0.0 | 13.9 ± 0.0 | 0.40 ± 0.1 | 5.9 ± 0.2 | 380 ± 14 |
| | 40 | 0.77 ± 0.1 | 0.1 ± 0.0 | 17.5 ± 0.9 | 0.50 ± 0.1 | 4.3 ± 0.1 | - |
| | 50 | 0.49 ± 0.0 | 0.1 ± 0.0 | 15.8 ± 0.5 | 0.45 ± 0.1 | 3.9 ± 0.1 | 0 ± 0 |

Note: CCR: combined chlorine residual; −: not determined; values are average ± standard deviation (n = 3).

**Table 3.** Concentration of DBPs in chlorinated Angaston WWTP effluent at a range of chlorine doses and 30 min contact time.

| Concentration of DBPs | Chlorine dose (mg $L^{-1}$) | | |
|---|---|---|---|
| | 15 | 30 | 50 |
| Nitrosamines (ng $L^{-1}$) | | | |
| *N-nitrosodimethylamine* | 38.7 ± 5.5 | 36.1 ± 0.6 | 28.3 ± 0.7 |
| THMs (µg L−1) | | | |
| Bromodichloromethane | <1.0 | <1.0 | <1.0 |
| Bromoform | <1.0 | <1.0 | <1.0 |
| Chloroform | 32.0 ± 0.0 | 1.0 | 3.0 ± 0.0 |
| Dibromochloromethane | <1.0 | <1.0 | <1.0 |
| Total THMs | 32.0 ± 0.0 | <4.0 | <4.0 |
| HAAs (µg L−1) | | | |
| Bromoacetic acid | <1.0 | <1.0 | <1.0 |
| Bromochloroacetic acid | 1.0 ± 0.0 | 2.0 ± 0.0 | 2.0 ± 0.0 |
| Bromodichloroacetic acid | <1.0 | <1.0 | <1.0 |
| Chloroacetic acid | <3.0 | <3.0 | <3.0 |
| Dibromoacetic acid | <1.0 | <1.0 | <1.0 |
| Dibromochloroacetic acid | <1.0 | <1.0 | <1.0 |
| Dichloroacetic acid | 4.0 ± 0.0 | 6.0 ± 0.0 | 7.0 ± 0.0 |
| Tribromoacetic acid | <1.0 | <1.0 | <1.0 |
| Trichloroacetic acid | 7.0 ± 0.0 | 9.0 ± 0.0 | 9.0 ± 0.0 |
| Total HAAs | 12.0 ± 0.0 | 17.0 ± 0.0 | 18.0 ± 0.0 |

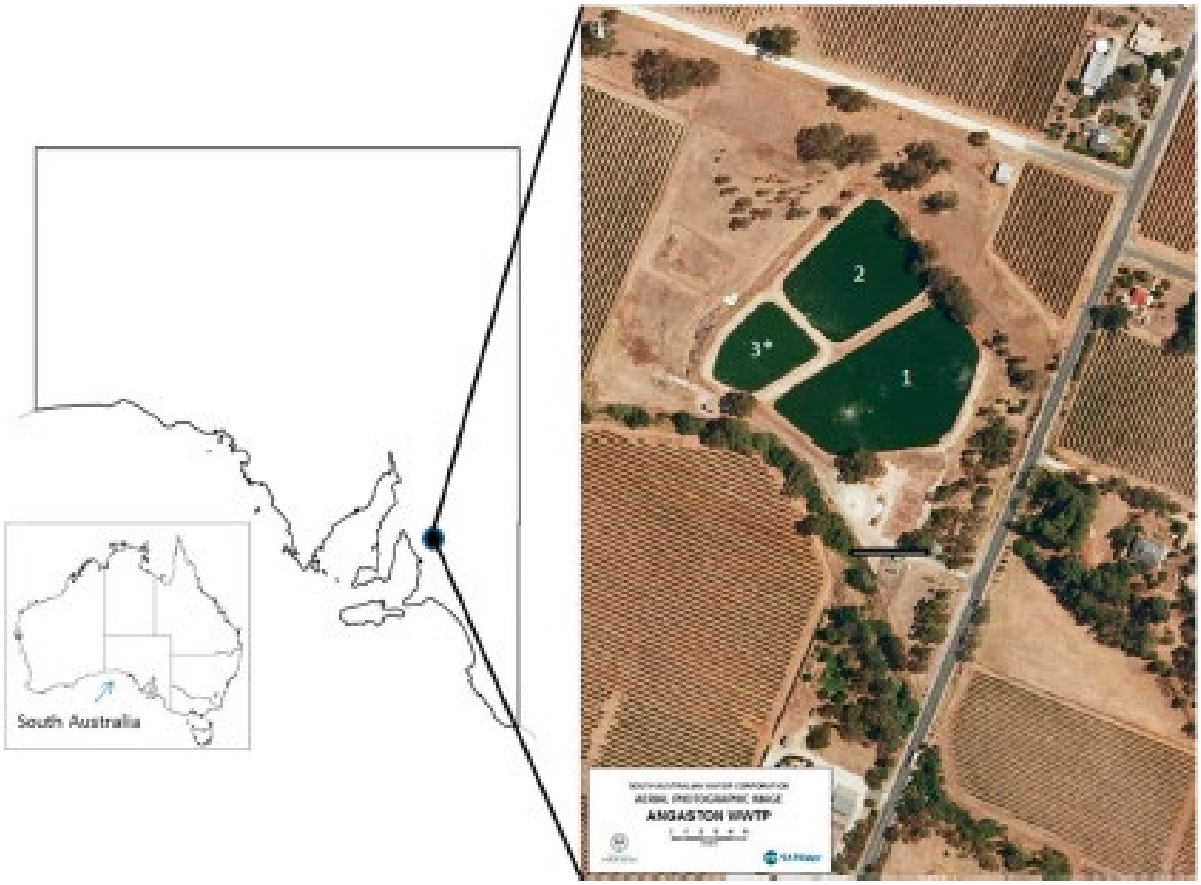


**Figure 1.** Geographic location of the WWTP in Angaston, South Australia, Australia. Order of WSP treatment is indicated by the numerals, asterix marks the WSP where the R. elegans bloom occurred (image courtesy of spectre visuals).

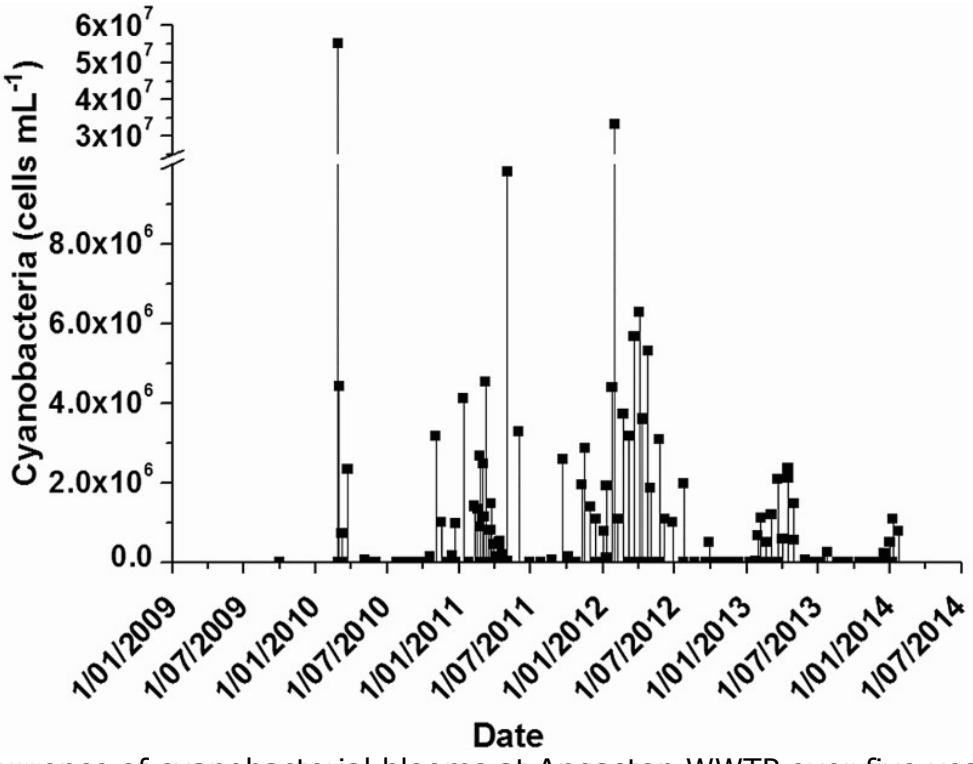


**Figure 2.** Occurrence of cyanobacterial blooms at Angaston WWTP over five years.

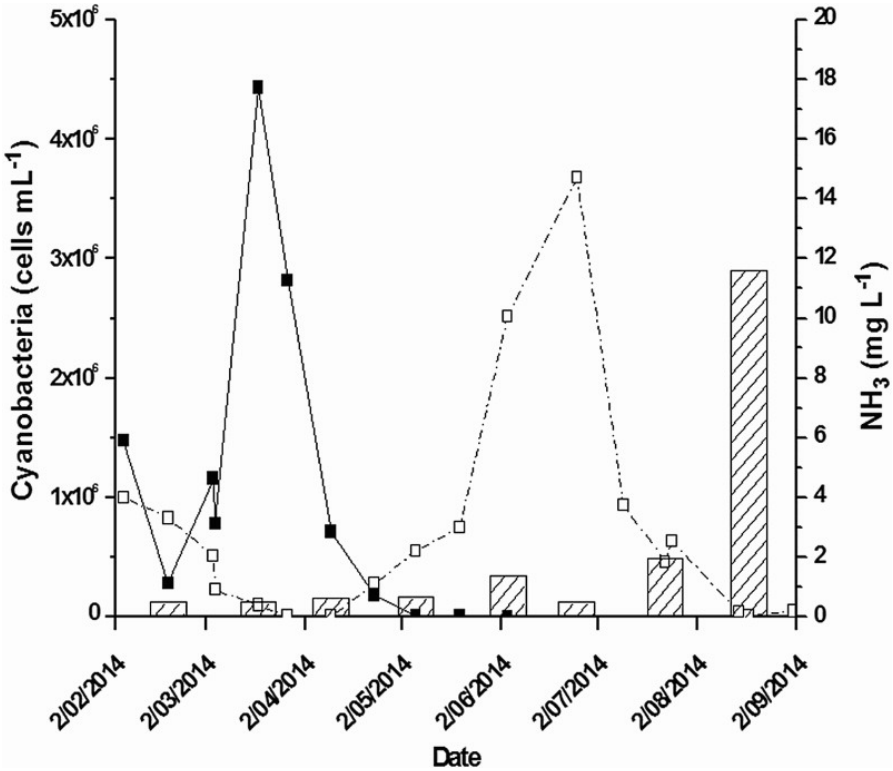


**Figure 3.** Relationship between the occurrences of the summer Microcystis sp. (▪) and the winter R. elegans (▫) blooms with the concentration of free ammonia (NH3) (columns) in the effluent of WSP 3 at Angaston WWTP.

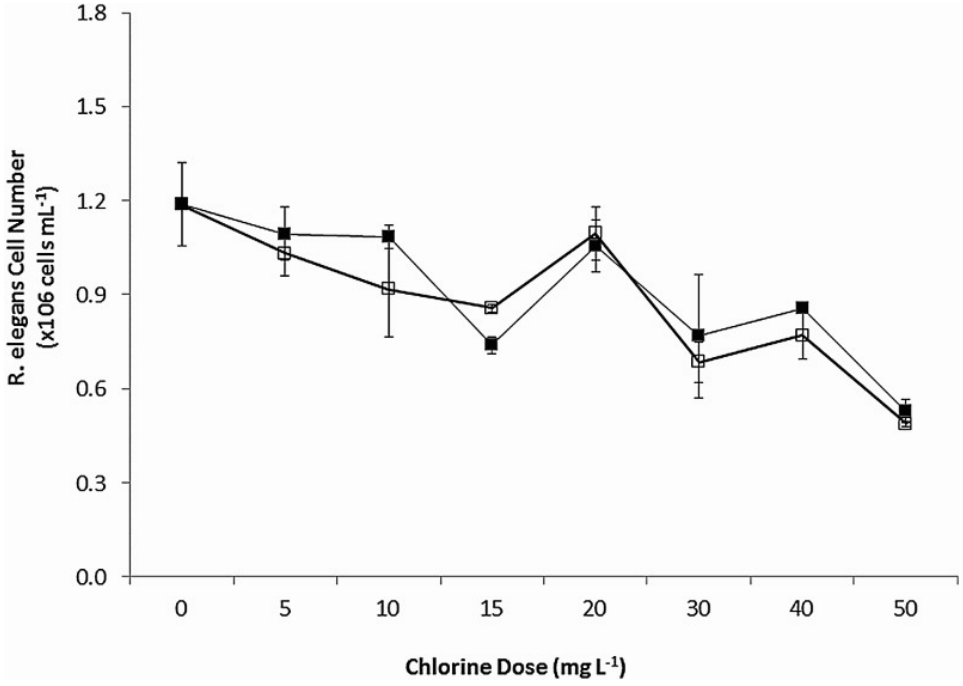


**Figure 4.** Reduction of R. elegans cell number during chlorination at different chlorine doses measured at 5 min (▪) and 30 min (▫) contact time (n = 3; error bars denote standard deviation).

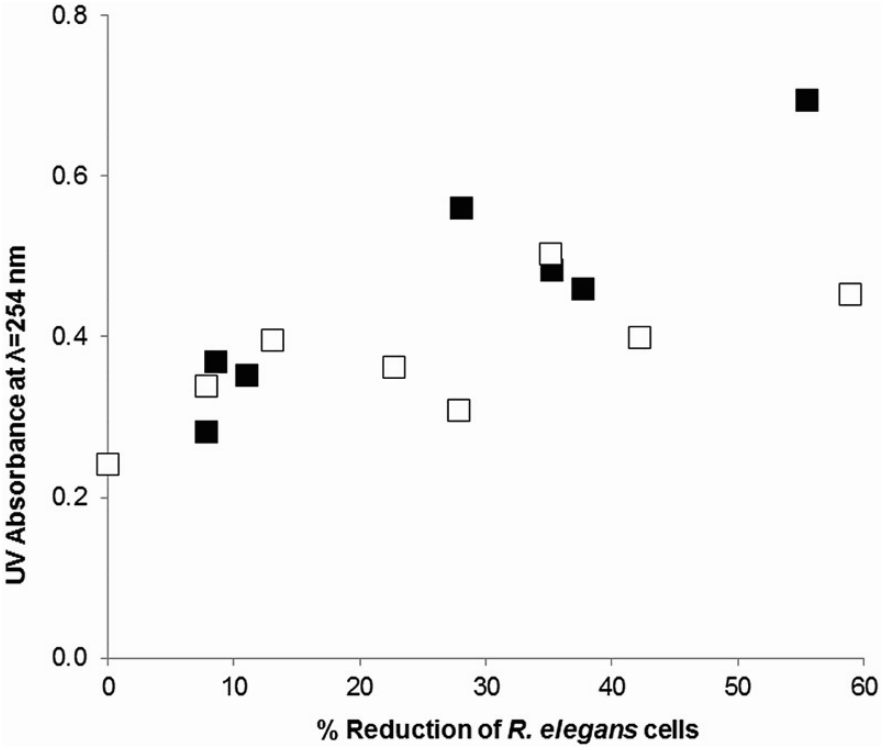


**Figure 5.** Release of algal IOM measured by increase of UV absorbance, as a result of cyanobacteria cell death during chlorination measured at 5 min (▪; R2 = 0.83) and 30 min (▫; R2 = 0.51) contact time.

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