Final Report

Project Title: “Reducing and Monitoring Blue-Green Algal Blooms”

Project Number: 62M-2026

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Executive Summary

Melbourne Water’s Western Treatment Plant (WTP) treats 52% of the wastewater from the metropolitan area of Melbourne and 72% of the industrial wastewater. After passing through a sequence of biological processes, including lagoon treatment, a portion of the water is disinfected and released as Class A recycled water. During the warmer months blooms of cyanobacteria (commonly known as blue-green algae), including Microcystis aeruginosa, periodically occur within the lagoons and can lead to interruption of the supply of recycled water from the affected lagoon system due to the presence of the toxin microcystin.

Recently, equipment suitable for the detection, prevention and reduction of nascent blooms has become available. These comprise on-line fluorometers which can detect algae and distinguish between blue-green and other algal classes, field use “dipstick” methods for the detection of algal toxin, ultrasonic algal controllers and solar-powered mixers which prevent blue-green algal blooms via habitat disturbance. Being relatively new on the market, these items have little operational history and thus little validation of effectiveness.

The overall aim of this project was to develop an increased understanding of the effectiveness of this equipment and so develop a robust system which provides a multi-barrier approach to the prevention, detection and reduction of blue-green algal blooms, and the treatment of any residual microcystin toxin.

The objectives of this work were:

- Comparison of phytoplankton and phytoplankton populations in the presence and absence of the SolarBee Unit installed at WTP.
- Investigation of the ability of the Algae Online Analyser (AOA) at WTP to quantitate suspensions of green and blue-green algae alone and in combination in the lagoon-treated water.
- Validation of Abraxis Microcystin Strip Test for the detection of microcystin in the lagoon treated effluents from WTP.
- Investigation of the impact of sonication on algal cells.

This report focuses on the key findings obtained from work completed over the period January 2009-October 2011 which are summarised below.

1 Comparison of phytoplankton and zooplankton populations in the presence and absence of the SolarBee unit

- Similar trends for the physicochemical parameters of Ponds 47 (with the SolarBee unit) and 56 (without the SolarBee unit) were observed. Despite sharing the same supply carrier, the phytoplankton and zooplankton populations differed significantly in the inputs to the ponds, making their direct comparison somewhat difficult.

- No clear trends for cyanobacterial populations were apparent over the monitoring period. After the installation of the SolarBee unit in Pond 47 in February 2009, there was a higher incidence of increased cyanobacterial populations in Pond 56, but not Pond 47. However, the benefit of mechanical mixing to control the cyanobacterial populations was not apparent since large numbers of Cyanophyceae (most notably short filamentous Planktolyngbya sp., cf Microcystis and Pseudanabaena sp. which have significantly large cell volumes) were present in the inlet and outlet samples of Pond 47 during February and March 2010 (cf means presumed).

- The literature suggested that artificial mixing may cause a shift in the species composition in a water body. As Ponds 47 and 56 are only 1.2 m deep, and according to DO and temperature data show minimal stratification, artificial mixing such as with the SolarBee unit may not reduce the mean light exposure of cyanobacteria to a level that would diminish their advantage of outcompeting other phytoplankton via self-regulated buoyancy, despite the high turbidity.
2 Evaluation of the Performance of the bbe Algae Online Analyser at WTP

- The AOA gave a consistent direct linear response to concentration (ranges covered 23,000-94,000 cells mL\(^{-1}\)) of *M. aeruginosa* (R\(^2\) values of at least 0.985). However, the measurement of chlorophyll \(a\) (Chl-\(a\)), and thus of the cyanobacterium, became increasingly less sensitive compared with a spectrophotometric method at cell concentrations greater than 50,000 cells mL\(^{-1}\). This was attributed to the shading or screening effect of the higher cell concentrations in the *in vivo* fluorometric method.

- The AOA cuvette was subject to fouling; although the correlation between cell concentration and Chl-\(a\) readings remained high, lower Chl-\(a\) readings resulted indicating apparently lower cyanobacterial concentrations. This is exemplified by the loss of response (approx. 20% on average) for the concentration range of 23,000-94,000 cells mL\(^{-1}\) over one month, however the response could be restored by cleaning with 12.5% (v/w) sodium hypochlorite.

- The presence of the green algae *Chlorella* sp. or *Euglena gracilis* influenced the response of the AOA to concentration of *M. aeruginosa* over the range of interest at WTP (35,000-65,000 cells mL\(^{-1}\)). When *Chlorella* or *E. gracilis* at a Chl-\(a\) level of 5.2 \(\mu\)g L\(^{-1}\) were added to suspensions of the cyanobacterium, the presence of *Chlorella* decreased the apparent Chl-\(a\) concentration for *M. aeruginosa* by an average of approximately 12% while the presence of *E. gracilis* increased the apparent Chl-\(a\) concentration by an average of approximately 20%. When *M. aeruginosa* was present at constant cell concentrations of 25,000 and 50,000 cells mL\(^{-1}\), in the presence of increasing concentrations of *E. gracilis* the apparent cell concentration increased, but remained the same after an initial decrease for *Chlorella*. This demonstrated that the AOA can give an overestimate or underestimate of *M. aeruginosa* populations when green algae are present in HORS water, depending on the relative numbers and species of the green algae.

- For populations of *M. aeruginosa* in the range 23,000-94,000 cells mL\(^{-1}\), the relative standard deviation for concentration of Chl-\(a\), and thus of apparent cell concentration, measured by the AOA was 10-12%.

- The results demonstrated that the AOA can be used as an alert system for cyanobacterial blooms in the HORS pond. However, the effect of the presence of some green algae on the apparent concentration of *M. aeruginosa* needs to be taken into account, the accuracy of the AOA in terms of Chl-\(a\) concentration should be cross-checked periodically with a laboratory-based method such as spectrophotometry, and calibration for yellow substances over an appropriate range needs to be maintained. As the deposition of particles, organisms and organics within the cuvette can negatively impact its performance, it is recommended that an automatic chemical cleaning system be installed to maintain consistent performance. Although the readings may be affected by these factors, the AOA provides real-time monitoring of algal populations and an indication of change in cyanobacterial numbers, e.g., imminent blooms. Ease of handling and relatively low maintenance costs are further advantages compared with manual cell counts which are time-consuming, and the accuracy of which is dependent on the sampling time, sample management and operator error.

3 Validation of the Abraxis Microcystin Strip Test for the detection of microcystin in the lagoon treated effluents from WTP.

- The Abraxis test strips gave a reliable indication of the concentration of microcystin LR (mLR) in a wide range of water matrices, i.e., those with organic components of different types and concentrations, and with different inorganic content (as measured by conductivity).

- The presence of *M. aeruginosa* cells did not appear to interfere with the detection of mLR in the lagoon-treated effluent.

- Interpolation of mLR concentrations within the reference range provided by the manufacturer of the strips tended to be subjective and give over-estimates, and, as the test strips give microcystin
concentrations in terms of mLR (the most toxic microcystin congener), the indication of toxicity tends to be high, and so conservative.

4 Investigation of the impact of sonication on algal cells

- Cell suspensions grown in artificial media were subjected to sonication (20 kHz) at varying power intensities (0.043-0.32 W mL\(^{-1}\)).

- Sonication led to an immediate reduction in the number of *M. aeruginosa* cells, with the initial 5 min of exposure giving the highest reduction rate. Increasing sonication power and exposure time resulted in greater immediate reduction in cell numbers and greater inhibition of the growth of the remaining *M. aeruginosa* cells over 7 days incubation.

- Sonication for 5 min at 32 W mL\(^{-1}\), or for a longer time (> 10 min) at a lower power intensity (0.043 W mL\(^{-1}\)), led to an immediate increase in microcystin levels in the treated suspensions. However, prolonged exposure (> 10 min) to sonication at higher power intensities reduced the microcystin concentration, which was attributed to ultrasonic degradation. Hence, the choice of suitable dosage for controlling the growth of *M. aeruginosa* should be a trade-off between the immediate and longer term sonication effects, release of toxin and power efficiency.

- Under the same sonication conditions, the order of decreasing growth inhibition for the species studied in this work was: *Anabaena circinalis > M. aeruginosa > Chlorella* sp., consistent with selective inhibition of cells possessing gas vacuoles by sonication. This demonstrates that sonication has the potential to selectively remove/deactivate cyanobacteria in wastewater treatment lagoons.

- Field scale studies should be conducted to verify the lab scale results and so provide more data for feasibility assessment and optimisation.
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1. Background

In the warmer months blooms of the toxic cyanobacterium *Microcystis aeruginosa* (commonly known as a blue-green alga) occur periodically within the lagoons at the Western Treatment Plant (WTP). These blooms can lead to interruption of the supply of recycled water to customers from the affected lagoon system due to the presence of the toxin microcystin which is considered to pose a risk to the health of the consumers of certain crops irrigated with it if eaten unwashed (eg., lettuce), as well as agricultural workers exposed to the recycled water.

Recently, equipment suitable for the detection, prevention and reduction of nascent blooms has become available. These comprise on-line fluorometers which can detect algae and distinguish between blue-green and other algal classes, field use “dipstick” methods for the detection of algal toxin, ultrasonic algal controllers and solar-powered mixers which prevent blue-green algal blooms via habitat disturbance.

Solar-powered long distance circulation technology has been developed as a cost-effective alternative to aeration in reservoir or wastewater lagoons (SolarBee, Inc., 2011). According to the manufacturer (SolarBee Inc.), long distance circulation not only provides the benefits of re-aeration at low cost but also prevents the occurrence of harmful cyanobacteria in nutrient-rich waters. The scientific basis for cyanobacterial control through circulation is described in the literature on habitat disturbance (Donaghay and Osborn, 1977; Reynolds at al., 1983; Jungo at al., 2001). The SolarBee solar-powered circulator floats on top of the reservoir/wastewater lagoon and water is drawn up from the bottom through an intake hose and spreads equally across the surface in a near laminar long-distance-flow pattern. The flow is created by a solar-powered motor which spins an axial flow impeller and the intake system allows a specific depth to be treated without disturbing the sediment. The horizontal and vertical epilimnetic mixing from the circulator reduces stratification and so disrupts the ability of the cyanobacteria to form blooms. In order to evaluate the performance of the solar-powered circulator at WTP, a water quality monitoring program was established in this project.

The traditional method of monitoring cyanobacterial blooms in drinking water sources has been by the collection of water samples which are sent to the laboratory for microscopic enumeration and chlorophyll measurement by spectrophotometry. This usually takes some days before the data becomes available. Another technique, “in vivo fluorometry”, for detection and measurement of algal populations, is based on the measurement of the fluorescence of chlorophyll in the live algal cells. No special sample handling or processing is required and thus in vivo fluorometry using on-line instrument systems is useful for profiling and for real-time data collection. The Algae Online Analyser (AOA, bbe Moldaenke, Kiel, Germany) is a fluorometer which can be used to differentiate the different classes of algae in water according to their excitation spectra. Previous studies showed that the AOA is a useful tool for monitoring potentially toxic *M. aeruginosa* blooms in water reservoirs (Izydorczyk et al., 2009).

Microcystins are hepatotoxic cyclic heptapeptides produced by cyanobacteria. Analysis of microcystin is most commonly carried out using high performance liquid chromatography (HPLC) methods (Lawton et al., 1994; Meriluoto et al., 2000). However, laboratory analysis of microcystins is time consuming, and requires sample processing to concentrate the microcystins and eliminate matrix contaminants (McElhiney and Lawton, 2005). Therefore, a semi-quantitative test which can be used in the field to determine the toxin levels in water sources would be useful for recycled water management.

Immunochromatography can be utilised as a rapid qualitative method for the detection of a range of algal toxins (Metcalfe and Codd, 2003) and an immunoassay kit for microcystins based on ADDA antibodies (Fischer et al., 2001) has been developed by Abraxis LLC (Pennsylvania, USA). The Abraxis Microcystins Strip Test kit uses a rapid immunochromatographic test for the detection of microcystins and nodularins in water. The test is based on the recognition of microcystins and nodularins and their congeners by specific antibodies. The toxin conjugate competes with the toxins in the sample for antibody binding sites. Abraxis kits have a limit of detection of 0.5-1 ppb with two types of kits available reading up to 5 or 10 ppb (levels of concern are >1 ppb in drinking and recreational water), and semi-quantitation is possible.

The Abraxis system can be used to measure total microcystins, i.e., extracellular plus intracellular toxin, and thus to gain an indication of the concentration of toxin which could be potentially released if the cyanobacterial cells were disrupted. The Abraxis strip test has been designed for field use and gives results within an hour. Some such tests are available for determining the safety of drinking water sources and recreational water for swimming. However, none have been marketed for testing the treated municipal
wastewater in which the higher concentrations and wider ranges of organic and inorganic solutes present may interfere with the test system and yield inaccurate results.

Ultrasound has recently been recognised as a method for controlling cyanobacterial blooms and is considered a pollution-free “green chemical technique” (Ma et al., 2005). Ultrasound at a high intensity is known to induce disruption of gas vesicles (or vacuoles), inhibit photosynthesis and destroy the membranes of cyanobacteria (Lee et al., 2001). The main effect of ultrasound on bloom control has been focused on the sedimentation of cyanobacteria through the breakdown of gas vesicles (Nakano et al., 2001). Sedimented cyanobacteria are unable to photosynthesise due to insufficient light reaching the sediment. In addition to disrupting the gas vesicles, sonication was also found to disturb the cell cycle and cell division; application of ultrasound late in the day was found to be most effective in reducing the growth rate of cyanobacteria as it corresponds to the daily cell division phase (Ahn et al., 2003). Although ultrasonic irradiation can cause microcystin to be released from cyanobacterial cells to water, it also can effectively decompose the microcystins dissolved in water (Ma et al., 2005). Ultrasonic power and frequency are important parameters for inhibition of cyanobacterial growth. The amount of energy input and costs should be considered if the ultrasonic method is to be put to practical use.

Being relatively new on the market, these equipment items have little operational history and thus little validation of effectiveness. To date, most experience has been with their application to drinking water. Comprehensive evaluation of the performance of this equipment in wastewater treatment is not yet available.

2. Description of Project

Melbourne Water installed a SolarBee circulator and an algae online analyser (AOA) at the Western Treatment Plant (WTP). The aim of this project was to develop an increased understanding of the effectiveness of the SolarBee circulator, the AOA and the Abraxis strip test, and the potential of sonication, so that a robust system which provides a multi-barrier approach to the prevention, detection and reduction of blue-green algal blooms could be developed for WTP.

The objectives of this work were:

- Evaluation of water quality data, phytoplankton and zooplankton populations at the inlet and outlets of Pond 47 (with SolarBee mixer) and Pond 56 over the period January 2009-November 2010 with a view to determining the efficacy of the SolarBee mixer for preventing cyanobacterial blooms.
- Investigation of the ability of the bbe AOA at WTP to quantitate suspensions of green and blue-green algae alone and in combination.
- Validation of the Abraxis Microcystin Strip Test for the detection of microcystin in the lagoon- treated effluent from WTP.
- Investigation of the impact of sonication on cyanobacterial cells.
3. Samples and Methods

3.1 Evaluation of the impact of the SolarBee circulator

Water samples were collected from the inlet and outlet of Ponds 47 and 56 periodically by Ecowise Environmental (later renamed ALS Laboratory Group). Ponds 47 and 56 are part of the 145W lagoon system, they are adjacent to each other, of similar depth and retention time, and receive effluent from the same supply carrier. In addition to determining the algal and zooplankton counts, Ecowise/ALS measured the following physicochemical parameters:

- Temperature
- pH
- Turbidity
- Alkalinity
- Chemical oxygen demand (COD)
- Dissolved organic carbon (DOC)
- Dissolved Fe and total Fe
- Chlorophyll a and phaeophytin a

See Appendix for details of sampling times.

Dissolved oxygen (DO) and temperature profiles of the ponds were determined on 25th March 2010 by Melbourne Water.

3.2. Evaluation of the AOA

- **WTP water:** recycled water collected from the Head of Road Storage (HORS) pond on the day of testing.

- **Algal cultures:** *M. aeruginosa* (CS 566/01-A01, originally isolated from WTP) was obtained from CSIRO Microalgae Research Centre, Hobart. *E. gracilis* and *Chlorella* sp. (obtained from Southern Biological, Nunawading) cultures were maintained in artificial culture media as per suppliers’ directions. They were all grown at 22°C under filtered (0.45 µm) humidified aeration using a 16/8 hour light/dark cycle in an algal growth cabinet. The cells were counted at RMIT University, and then 1 L suspensions made up in HORS water on the day of the experiment at WTP.

- **Fluorometric determination of Chlorophyll a:** The Chl-a concentrations for the three organisms were measured using the AOA and spectrophotometry. The AOA system was flushed with the test cell suspension three times before measuring Chl-a concentration.

- **Spectrophotometric determination of chlorophyll a:** The algal suspensions were filtered (1 µm glass fibre, Whatman GF/B) and the Chl-a was extracted with 90% (v/v) acetone overnight. The absorbance of the extract was measured at 750, 663, 645 and 630 nm (Unicam UV2 spectrophotometer), and the Chl-a concentration was calculated according to ESS method 150.1 (1991).

3.3 Validation of the Abraxis Microcystin Strip Test

- **Test strips:** Abraxis Microcystin Strip Test Kits PN 520020 (for up to 5 ppb microcystin) and PN 520022 (for up to 10 ppb microcystin) were obtained from Abraxis LLC, Warminster, PA., USA. The strips were used according to the manufacturer’s directions for the determination of extracellular and intracellular microcystin.

- **Water matrices:** A range of water matrices was used in this study: laboratory tap water; 3 samples of lagoon-treated water from WTP collected on 30/09/09, 25/11/09 and 11/06/10; activated sludge-treated effluent collected from Eastern Treatment Plant (ETP), and Yarra River water collected at Eltham on 22/12/09.

- **M. aeruginosa:** The *M. aeruginosa* was cultured in lagoon-treated effluent from WTP. Although the samples for testing were obtained from the same stock culture, the experiments were done on
different days and so the age of the culture varied between tests, this may have influenced the intracellular concentrations of microcystin.

- **Analyses of water quality:** UV absorbance at 254 nm was determined using a Unicam UV2 Spectrophotometer. Conductivity and pH were measured with a Sension 156 pH/conductivity meter. Dissolved organic carbon (DOC) was measured with a total organic carbon analyser (Sievers 820). Fluorescent excitation-emission matrix spectra were obtained with a Perkin Elmer LS 55 Fluorescence Spectrometer with the excitation and emission wavelengths in the range of 200-600 nm. Samples were filtered (0.45 µm cellulose acetate membrane) to remove any suspended particulates prior to DOC and fluorescence measurement.

- **Microcystin standard:** Microcystin-LR (mLR) was obtained from Alexis Biochemicals (Axxora LLC, San Diego, CA).

- **Preparation of microcystin-spiked samples:** Water samples (100 mL), with and without the addition of *M. aeruginosa*, were spiked with mLR at various concentrations and incubated in the dark for 60 min at room temperature. For the measurement of total mLR, the QuikLyse procedure was utilised as per manufacturer’s directions on samples with added *M. aeruginosa*, as indicated, and the whole or lysed cells were removed by filtration (1 µm Whatman GF/B) and the filtrate subjected to solid phase extraction.

- **Solid phase extraction of microcystin:** Trifluoroacetic acid (TFA) and methanol were added to sample aliquots to give final concentrations of 0.1% (v/v) and 1%, respectively. A Vac Elut SPS-24 vacuum manifold system (Varian Analytical Instruments) was set up with C18 trifunctional, end-capped solid-phase extraction cartridges, 0.5 g in a 3 mL syringe (Bond Elut, Varian). The cartridges were conditioned using 10 mL methanol followed by 10 mL water. Water samples were applied to the cartridges and then the cartridge was washed with 10 mL of Milli-Q water and 30% v/v aqueous methanol. The cartridges were then dried by the passage of air and followed by elution with 5 X 200 µL of 75% methanol and 25% acidified Milli-Q water (0.1% v/v TFA).

- **HPLC analysis:** HPLC analysis was carried out using a Gilson Model GX-281 solvent-delivery system and a Gilson Model 156 UV-vis detector at 238 nm. A Merck Purospher® STAR C18 endcapped column (3 µm) (55 x 4 mm i.d.) was used. The injection volume was 20 µL. Separation was achieved using a linear gradient comprising water-0.05% v/v TFA (A) and acetonitrile (B) supplied at 1 mL/min as described in Table 1. The method was validated by spiking 7 x 100 mL samples of lagoon-treated water with 5.0 ppb mLR and conducting the solid-phase extraction and HPLC analysis. A result of 4.96 ppb with standard deviation of 0.22 ppb was obtained.

<table>
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<th>22</th>
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<td>Acetonitrile (%)</td>
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<td>70</td>
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<td>100</td>
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</tr>
</tbody>
</table>

### 3.4. Investigation of the impact of sonication on algal cells

- **Algae samples:** *M. aeruginosa* and *Chlorella* sp. were obtained and cultured as in Section 3.1; *Anabaena circinalis* was obtained from Southern Biological, Nunawading and cultured in artificial medium as per supplier’s directions.
• **Cell enumeration:** The optical density (OD) of the algal suspension at 684 nm was used as a means of monitoring cell concentration and hence cell growth. The OD was measured at 684 nm using a UV-vis spectrophotometer (Unicam UV2) with Milli-Q water as the blank.

• **Sonication:** The frequency of the lab sonicator (VC750, Sonics and Materials, USA) was similar to that of the ultrasonic algal controllers installed at WTP. The conditions of 0.043 and 0.32 WmL\(^{-1}\) were chosen for comparison with previous work (Zhang et al., 2006a; Joyce et al., 2010) and intermediate ultrasonic densities were used to study the progressive effect. For each sample, a concentration of 2 x 10\(^6\) cells mL\(^{-1}\) was used. The sample volume for ultrasoniciation was 200 mL placed in a 250 mL beaker, except for 0.043 WmL\(^{-1}\) where 310 mL was required to attain the power density (the lowest ultrasonic density that could be achieved in a 200 mL solution with the ultrasonic probe was 0.067 WmL\(^{-1}\)). The samples were sonicated by inserting the probe 3 cm into the algal suspension and the beaker was kept in an ice water bath to maintain a temperature of 20 ± 5°C. Each sample was sonicated for 5, 10, 15 or 20 minutes. The samples were allowed to stand for 30 minutes, after which 4 mL of the suspension was taken from 1 cm below the surface and the OD\(_{684}\) was measured. A control sample which was not exposed to sonication was used for each study to enable comparison of the effects of sonication on algal growth. The effect of sonication on subsequent growth was determined by incubating the sonicated cell suspensions under the same conditions as described above, and the OD\(_{684}\) was determined periodically for 168 h.

• **Measurement of microcystin:** Abraxis Microcystin Strip Test Kits as described in Section 3.3 were used. The extracellular microcystin was measured after removal of the cells by filtration (0.45 µm).
4. Key Activities Completed

4.1 Evaluation of the impact of the SolarBee circulator

The trends for the following parameters for Pond 47 (with the SolarBee circulator) and Pond 56 (without the SolarBee unit) for the period January 2009-May 2010 were determined:

- Water quality: temperature, turbidity, pH, alkalinity, DOC, COD, dissolved and total Fe
- Chlorophyll a and phaeophytin a
- Phytoplankton and zooplankton
- DO and temperature profiles (measured on 25th March 2010).

4.2 Evaluation of the AOA

- Response of the AOA to concentration of *M. aeruginosa* in HORS water.
- Comparison of the results obtained from the AOA with spectrophotometric method.
- The reproducibility of the AOA results.
- Effect of the green algae, *Chlorella sp.* and *E. gracilis*, on the response of the AOA to concentration of *M. aeruginosa*.
- Effect of chemical cleaning on the response of the AOA to concentration of *M. aeruginosa*.

4.3 Validation of Abraxis Microcystin Strip Test

- Comparison of results measured by Abraxis strips and HPLC for different water samples spiked with mLR.
- Determination of the influence of lagoon-treated water on Abraxis test results.
- Comparison of microcystin levels for Abraxis strip test and HPLC for lagoon-treated water containing various concentrations of *M. aeruginosa*.
- Determination of suitability of the Abraxis strip test for evaluation of elevated concentrations of microcystin.

4.4 Investigation of the impact of sonication on algal cells

- Impact of sonication at various power intensities on cell growth and microcystin release by *M. aeruginosa*
- Impact of sonication on the growth of other algal species: *Chlorella sp.* and *A. circinalis*. 
5. Results Achieved

5.1 Evaluation phytoplankton and zooplankton populations in the presence and absence of the SolarBee unit

5.1.1 Trends for water quality

- The general trend for temperature was consistent with seasonal variations in both ponds during the sampling period (Appendix - Figures A1 and A2). The average temperature during the warmer seasons was about 20°C, although 25°C was recorded on 27th January 2009. In winter, the lowest water temperature was 9°C (on 16th June 2009). The turbidity of the samples usually ranged between 10 and 30 NTU, but occasionally readings as high as 79 NTU were reported. In many cases of higher turbidity, the samples were collected later in the day (i.e., after 9 a.m.). The pH of the inlet and outlet samples of the ponds was fairly consistent, usually between pH 8.0 and 8.5, with an average of pH 8.3. An exception was for the inlet of Pond 56 on 15th May 2009 which recorded the lowest pH of 7.2. The alkalinity for the pond inlets and outlets followed similar trends, indicating that the source water was responsible for the observed fluctuations. There was an average alkalinity of 300-360 mg CaCO₃ L⁻¹, with a trend to increased levels during the colder seasons. This may be associated with the reduced photosynthetic activity of algae accompanying their lower uptake of CO₂, and the release of CO₂ from bacterial respiratory processes being converted to alkalinity under higher pH conditions.

- The DO and temperature profiles of the ponds as measured on 25th March 2010 indicated minimal stratification in both ponds, however the DO in Pond 47 was on average higher than in Pond 56 (approximately 6 compared with 4 mg L⁻¹, respectively, Appendix - Figure A3).

- No correlation between the changes in COD and DOC were apparent (Appendix - Figures A4 and A5). There was a tendency toward increasing inlet and outlet DOC concentration for Pond 47 (with SolarBee unit), whereas it was more consistent for Pond 56. The COD of the samples generally ranged from 5 to 250 mg L⁻¹, the highest value of 450 mg L⁻¹ was recorded on 16th May 2010 for the inlet of Pond 47. The fluctuation in COD appeared to be synchronised with that of turbidity, suggesting that the component(s) that led to increased turbidity also contributed to the COD.

- Chlorophyll a and phaeophytin a concentrations appeared to fluctuate synchronously with COD and turbidity (Appendix - Figures A6 and A7). There was a moderate to strong relationship between the COD and chlorophyll a concentration (Appendix - Figure A8) with R² values of 0.68 to 0.87. The linear relationship shown in Figure A8 indicated that every mg L⁻¹ of chlorophyll a corresponded to 91-109 mg L⁻¹ COD, and if the concentration of chlorophyll a were zero, other substances in the water matrix would contribute 74-78 mg L⁻¹ of COD to the samples.

- Phaeophytin a is often used to indicate algal health as increasing concentrations are associated with an ageing population (Panova and Dimkov, 2008). The chlorophyll a concentration of the samples was observed to be 3.3 to 4.3 times that of phaeophytin a (Appendix - Figure A9). While this translated to a substantial degradation of chlorophyll a (about 18-23%), it may indicate not only the physiological condition of the algal population but also reflect the effectiveness of sample handling and preservation (Chorus and Bartram, 1999). According to Louda et al. (1998), the ratio of phaeophytin a to chlorophyll a varied considerably for different algal cultures and can be impacted by various ageing processes.

- The concentrations of soluble and total iron in the inlet and outlets of the ponds were fairly similar (Appendix - Figures A10 and A11). There was no seasonal trend for soluble and total iron concentrations, and average values were 0.1 and 0.4 mg L⁻¹, respectively.

5.1.2 Trends for total algal counts

- In 2009, the total algal counts were mostly less than 20,000 cell units mL⁻¹ and the phytoplankton were largely categorised as Chlorophyceae and Euglenophyceae (Figure 1). Chlorophyceae were generally present in higher numbers in Pond 56 than in Pond 47 (with SolarBee). While Cyanophyceae were occasionally abundant, other phytoplankton described as “small unidentified flagellates” were present in very large numbers, boosting the total algal count to much higher than 50,000 cell units mL⁻¹ on several occasions (27th Jan, 13th Mar, 17th Mar and 14th September). The occurrence of these unidentified flagellates appeared to be random, and may be a result of carryover...
from preceding lagoons or production *in situ*. There was an influx of *Cryptophyceae* to Pond 47 on 17th August although their numbers dwindled within a month.

![Graph showing algal count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009. Shading of sample collection date refers to collection time, see Appendix for details.]

- The data for 2010 were a little different (Figure 2). As in 2009, *Chlorophyceae* populations were generally higher in the inlet and outlet of Pond 56 than Pond 47. However, in comparison with 2009, there was a significant increase in the populations of *Euglenophyceae* and *Cyanophyceae*, especially for Pond 47. In many cases, the increases were for both the inlet and outlet of the ponds, indicating carryover from preceding lagoons.
Figure 2. Total algal count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.

5.1.3 Trends for the Cyanophyceae genera

The presence of Cyanophyceae is frequently associated with issues such as taste, odours, depletion of dissolved oxygen and toxicity. Some species, including Anabaena, Aphanizomenon and Microcystis, release neurotoxins or hepatotoxins which cause the poisoning of livestock and aquatic fauna (Kanitz and Franco, 2002).

While many algae are able to make depth adjustment according to changes in environmental stimuli, the cyanophyta adjust their buoyancy via control of gas vesicle gene expression, collapse of vesicles by alteration of turgor pressure, and formation of carbohydrate ballast by photosynthesis. Species such as Microcystis and Aphanizomenon have upward speeds of about 3 m h$^{-1}$ which greatly exceed the swimming speed of other mobile algae such as dinoflagellates and green algae (Sigee, 2004).

In addition to C, H, N, O and P, iron and manganese are required by algae for photosynthesis, nitrate assimilation and many enzymatic processes. The depletion of dissolved iron levels often coincides with the end of a bloom of cyanobacteria, such as Aphanizomenon flos-aquae, that are capable of fixing atmospheric nitrogen (Knappe et al., 2004). In Figures 3 and 4, the Cyanophyceae population in 2009 and 2010 (in particular Aphanocapsa sp.) are compared with the fluctuations in the concentrations of total and soluble iron, while Figures A16 and A17 (Appendix) illustrate the distribution of other blue-green algae. No consistent trend was found to correlate the Cyanophyceae blooms with the levels of total and dissolved iron; the elevated cyanobacterial count in 2010 appeared to occur during high concentration of total iron but this was not so in 2009 even though much higher levels of total iron were detected. The specific trends for the different cyanobacterial populations are discussed below.
Figure 3. Trend for soluble and total iron compared with the *Cyanophyceae* and *Aphanocapsa sp.* counts for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure 4. Trend for soluble and total iron compared with the *Cyanophyceae* and *Aphanocapsa sp.* counts for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
5.1.3.1 Trends in Cyanobacterial counts for 2009

Analysis of the data yielded the following trends and comments:

- Before installation of the SolarBee unit in Pond 47 in February 2009, a cyanobacterial bloom of about 16,000 cell units mL\(^{-1}\) occurred on 21\(^{st}\) January 2009; it appeared to be produced in situ rather than as a result of carryover from a preceding lagoon. Aphanocapsa sp. contributed to about 40% of the Cyanophyceae and this was the only incidence of filamentous Pseudanabaena sp. occurring in significant numbers over the entire monitoring period (Appendix - Figure A17).

- On 11\(^{th}\) March 2009, more than 10,000 cell units mL\(^{-1}\) of Cyanophyceae were present at the inlet of Pond 47 but this reduced to about 6,000 cell units mL\(^{-1}\) at the outlet; this was accompanied by a period of lower dissolved iron levels. Aphanocapsa sp. and Planktolyngbya sp. were present in almost equal proportions. On the same day, the Cyanophyceae population was about two times higher in the inlet of Pond 56 and up to 18,000 cell units mL\(^{-1}\) was recorded in the outlet. Planktolyngbya sp. presented in significant numbers (although lower than for Pond 47) but Aphanocapsa sp. were predominant.

- An increase in Cyanophyceae occurred on 31\(^{st}\) March 2009 in the inlet of Pond 56. At 4,000 cell units mL\(^{-1}\) (mainly Pseudanabaena sp. cells), it did not go on to form a cyanobacterial bloom in the pond as evidenced by the Cyanophyceae counts for the outlet on the 3\(^{rd}\) and 7\(^{th}\) April 2009.

- The next cyanobacterial bloom occurred on 9\(^{th}\) April 2009 in Pond 56. Aphanocapsa sp. were present almost exclusively, and they were detected at levels of up to 53,000 cell units mL\(^{-1}\) in the outlet sample only. Since both ponds have comparable physicochemical conditions (apart from lower COD level in the outlet sample of Pond 56), it may be possible that the operation of the Solarbee unit prevented a similar bloom in Pond 47.

- For the remainder of 2009, Cyanophyceae populations were not present in substantial numbers in Pond 47. For Pond 56, however, Cyanophyceae populations of 5,000–7,500 cell units mL\(^{-1}\) were reported for 16\(^{th}\) June and 14\(^{th}\) September 2009; filamentous Planktolyngbya sp. and Aphanocapsa sp. were the dominant species in the respective episodes.

Assuming that Ponds 47 and 56 experienced similar conditions, the 2009 data suggest that the SolarBee unit successfully controlled or prevented the bloom of blue-green algae. The inconsistency of sampling time (Appendix-Table A1) would not significantly impact the interpretation of these data as almost all samples with substantial Cyanophyceae counts were collected before 9 a.m.

5.1.3.2 Trends in Cyanobacterial counts for 2010

Examination of the 2010 data (Figure 4) showed that there were more cyanobacterial bloom incidents than in 2009 (Figure 3), the Cyanophyceae cell counts were mostly higher for Pond 47 (with SolarBee) than Pond 56, and there was a mixture of sampling times when high numbers of Cyanophyceae were recorded. These can be summarized as follows:

- About 3,000 cell units mL\(^{-1}\) of Cyanophyceae entered Pond 47 and 56 on 14\(^{th}\) January 2010 and almost double those numbers were found in the outlets. The populations were a mixture of Aphanocapsa sp., Planktolyngbya sp. and Pseudanabaena sp. (Appendix - Figure A17).

- For Pond 47, the Cyanophyceae population started to increase on 9\(^{th}\) February and was 3,000 to 6,000 cell units mL\(^{-1}\) till 9\(^{th}\) March. The numbers then fluctuated between 10,000 and 60,000 cell units mL\(^{-1}\) until 21\(^{st}\) April 2010.
  - During this period, Aphanocapsa sp. was present in significant numbers at the inlet of the pond on 16\(^{th}\) March but did not survive for more than 8 days in the pond. On the same day, about 6,000 cell units mL\(^{-1}\) of Cyanophyceae that resembled cf. Microcystis entered the pond, and more than 14,000 cell units mL\(^{-1}\) were found in the outlet sample. (According to ALS Laboratory cf means presumed).
  - Merisopedia sp. (not reported in 2009) entered the pond for the first time on 1\(^{st}\) March 2010 and although they exceeded 12,000 cell units mL\(^{-1}\) on 9\(^{th}\) March 2010, the population soon declined.
  - Geitlerinema sp. was detected intermittently and the conditions in the pond apparently did not sustain its growth.
Although found regularly in the inlet samples of Pond 47, the higher numbers of *Pseudanabaena* sp. (cells) in the outlet samples indicated that the SolarBee unit may not have substantial impact on controlling their populations.

Filamentous *Planktolyngbya* sp. was the main contributor to the *Cyanophyceae* found at both the inlet and outlet of Pond 47. While they were described as a mixture of short and long filaments by the biologists of Ecowise/ALS, the latter was almost negligible proportionally.

- The population of *Cyanophyceae* entering Pond 56 also started to increase in mid February 2010 (to 5,500 cell units mL\(^{-1}\), Appendix - Figures A15 and A17). In March, the numbers increased to 20,000 and then to 77,000 cell units mL\(^{-1}\). The population of *Cyanophyceae* detected in the outlet of the pond increased correspondingly, but to a lesser extent, and remained at about 3,500 cell units mL\(^{-1}\) until the end of April.

- Up to 19,000 cell units mL\(^{-1}\) of *Aphanocapsa* sp. entered Pond 56 in March, and similar to Pond 47, they were not found in large numbers in the outlet samples.

- *Merisopedia* sp. also entered Pond 56 for the first time on 1\(^{st}\) March, but in much higher numbers compared with Pond 47. At 49,000 cell units mL\(^{-1}\) in the inlet on 9\(^{th}\) March, it was the predominant *Cyanophyceae* species in Pond 56. Although large numbers entered the pond, the population of *Merisopedia* sp. soon diminished and the organisms were detected only intermittently in the subsequent samples.

- *Geitlerinema* sp. entered Pond 56 occasionally but did not seem to establish a population in the pond.

- *Pseudanabaena* sp. (cells) was found in several inlet samples of Pond 56. Similar to Pond 47, these blue-green algae grew to produce substantial populations, suggesting that the mechanical mixing provided by the SolarBee (or lack thereof) does not affect their growth.

- In contrast to Pond 47, filamentous *Planktolyngbya* sp. were found only in low numbers in inlet samples for Pond 56 and populations did not become established in the pond.

As of 8\(^{th}\) December 2009, the biovolumes of *Cyanophyceae* were reported (Table 2). *Aphanocapsa*, the predominant species in several cyanobacterial blooms in 2009 (Figure 3), have small cell volumes of 1.26 \(\mu\)m\(^3\), whereas *Merismopedia* sp. (mainly small cells), present in large numbers in Pond 56 in 2010 (Figures 17 (b) and (d)), were the smallest (0.50 \(\mu\)m\(^3\)). In contrast, short filamentous *Planktolyngbya* sp. which was abundant in Pond 47 (Figures 17 (a) and (c)) has a cell volume of 30 \(\mu\)m\(^3\), whereas cf *Microcystis* and *Pseudanabaena* sp. (cells) have cell volumes of 113 and 12.5 \(\mu\)m\(^3\), respectively.

The impact of the differences in cell volumes and cell numbers amongst the various cyanobacteria is clearly illustrated when the biovolume of *Cyanophyceae* is plotted (Appendix - Figures A18 and A19). In Pond 47, the biovolume of short filamentous *Planktolyngbya* was unmistakably high on several occasions. The long filamentous *Planktolyngbya* were of significant biovolume in samples collected on 8\(^{th}\) April 2010 and the biovolume of cf *Microcystis* was substantial for the samples collected on 16\(^{th}\) March 2010. Although *Phormidium* sp. (medium cells) did not present in large numbers (Appendix - Figures A16 and A17), they had a large cell volume (142 \(\mu\)m\(^3\)) and hence their biovolumes were noticeable in several samples. *Pseudanabaena* sp. were usually found to be less than 0.1 mm\(^3\) L\(^{-1}\). The biovolumes of various cyanobacteria were generally low in samples collected from the inlet and outlet of Pond 56. Except in one sample (9\(^{th}\) March 2010), for which the biovolume of short filamentous *Planktolyngbya* was relatively high (about 0.25 mm\(^3\) L\(^{-1}\)), the biovolume of *Pseudanabaena* sp. was less than 0.1 mm\(^3\) L\(^{-1}\).

While the biovolumes of cyanobacteria did not appear to correlate with the trends of other parameters, these results indicate that the cyanobacterial populations, especially in Pond 47 in 2010, were not only significant in terms of numbers, but also in terms of their biovolumes.

Sigee (2004) stated that the surface accumulation of blue-green algae requires the establishment of well-stratified static water conditions which are indicated by the formation of an epilimnion (oxygen-rich surface layer with high biomass of autotrophs and low inorganic nutrients), a metalimnion (transition layer where steep decrease in dissolved oxygen and light intensity are observed) and the lowest layer of the hypolimnion (oxygen-depleted zones with high nutrients and the concentration of heterotrophs). In the review by Oliver and Ganf (2000) on cyanobacterial blooms in freshwater lakes, it was suggested that the development of cyanobacterial blooms depend on several physical factors, in particular the degree of water column stability, when more than 10 \(\mu\)g L\(^{-1}\) total phosphorus is present. Unfortunately, total phosphorus data were not available for this project.
Table 2. The cell volumes of *Cyanophyceae* species found in Ponds 47 and 56 (according to data provided by Ecowise/ALS)

<table>
<thead>
<tr>
<th><em>Cyanophyceae</em></th>
<th>Cell Volume (µm$^3$)</th>
<th><em>Cyanophyceae</em></th>
<th>Cell Volume (µm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphanocapsa (cells)</td>
<td>1.26</td>
<td>Phormidium (medium cells)</td>
<td>142.00</td>
</tr>
<tr>
<td>Aphanothece (cells)</td>
<td>2.10</td>
<td>Phormidium (small cells)</td>
<td>98.00</td>
</tr>
<tr>
<td>cf Microcystis (single cells)</td>
<td>113.00</td>
<td>Planktolyngbya sp. (long filaments)</td>
<td>120.00</td>
</tr>
<tr>
<td>Geitlerinema sp. (cells)</td>
<td>23.00</td>
<td>Planktolyngbya sp. (short filaments)</td>
<td>30.00</td>
</tr>
<tr>
<td>Leptolyngbya (cells)</td>
<td>2.36</td>
<td>Planktolyngbya subtilis</td>
<td>5.89</td>
</tr>
<tr>
<td>Limnothrix</td>
<td>12.00</td>
<td>Planktolyngbya</td>
<td>3.80</td>
</tr>
<tr>
<td>Merismopedia sp. (medium cells)</td>
<td>11.00</td>
<td>Planktothrix sp. (small cells)</td>
<td>56.00</td>
</tr>
<tr>
<td>Merismopedia sp. (small cells)</td>
<td>0.50</td>
<td>Pseudanabaena sp. (cells)</td>
<td>12.50</td>
</tr>
<tr>
<td>Phormidium (large cells)</td>
<td>212.00</td>
<td>Synecococcus</td>
<td>46.00</td>
</tr>
</tbody>
</table>

Citing the work of Steinberg and Zimmermann in 1988, Oliver and Ganf (2000) indicated that artificial destratification can cause a major shift in species composition, and mixing regime (continuous versus intermittent) can result in the reduction of some cyanobacterial populations but encourage the growth of others. They used an example from the work of Steinberg and Gruhl in 1992 to illustrate how destratification of a relatively shallow lake (mean depth of 5.7 m) suppressed the growth of *Limnothrix* in the first two years. However, its subsequent reappearance led to the conclusion that “although the continuous mixing produced relatively constant mixing patterns with a low mean irradiance, the lake was not sufficiently deep to reduce the mean irradiance below that required by the *Oscillatoriaceae*”. This suggests that while mechanical mixing may, in theory, prevent the surface accumulation of blue-green algae and hence reduce their advantage over other phytoplankton to compete for light energy, the actual reduction in their light exposure must be lowered to below a certain threshold so that it would no longer sustain their growth, and this threshold may be specific to different species.

With an average depth of 1.2 m, the extent of stratification that would have occurred in Ponds 47 and 56 cannot be ascertained, however, the temperature and DO profiles determined by Melbourne Water on 25th March 2010 indicated there was minimal if any stratification at that time. The apparently conflicting results collected in 2009 and 2010, demonstrated by the significant increase in several populations of *Cyanophyceae* (including cf. *Microcystis*, *Pseudanabaena* sp., and filamentous *Planktolyngbya* sp. in some cases) in the outlet samples compared with those entering Pond 47 in 2010, the benefits of mechanical mixing on the control of cyanobacterial blooms in such a lagoon system remained inconclusive.

According to Oliver and Ganf (2000), McAuliffe and Rosich conducted a review in 1989 and found that artificial destratification failed to control phytoplankton in over 60% of 52 Australian reservoirs. In a more recent study, Carlson and Foster (2009) concluded that the deployment of 12 SolarBee units (model SB10000v12) in Copco reservoir in northern California “did not act to improve water quality, and in particular did not act to reduce cyanobacteria blooms”. This was in contrast to the claim by the manufacturer that the water circulators have been proven to be effective in over 200 water bodies worldwide.
5.1.4 Trends for the Chlorophyceae and Euglenophyceae

- The populations of Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae and Euglenophyceae found in the inlet and outlet of Pond 47 and 56 in 2009 and 2010 are presented in Figures A20 and A21. Chlorophyceae was regularly present in both ponds, and their numbers were mostly higher in Pond 56 than in Pond 47. Euglenophyceae were also plentiful, usually at less than 10,000 cell units mL\(^{-1}\), although their numbers increased greatly from mid February to mid April 2010. While this was mainly a result of carryover from the preceding lagoons, the conditions in Pond 47 during this period appeared to sustain their growth in many cases. Interestingly, this coincided with the cyanobacterial bloom observed in Pond 47.

- A closer examination of the Chlorophyceae population in Pond 56 revealed that Coelastrium sp. was usually the dominant species from 2009 (Figure A22 (c)-(d)) to February-March 2010 (Figure A25 (c)-(d)), after which it was overtaken by Pediasastrum sp. in Pond 47 (Figure A22 (a)-(b), Figure A25 (a)-(b)). Coelastrum sp. was also regularly present, but to a lesser extent compared with Pond 56; Pediasastrum sp. counts as high as 7,000 cell units mL\(^{-1}\) were found in some Pond 47 outlet samples prior to the installation of the SolarBee unit, but it cannot be ascertained if this was coincidental.

- Although in relatively lower numbers, Chlorococcoids and Oocystis sp. were detected in both ponds frequently, whereas the numbers of Closterium sp. and Selenastrum sp. spiked occasionally without showing a particular trend. Scenedesmus sp. were found in higher numbers in Pond 56 than in Pond 47; Chlamydomonas sp. were recorded in both ponds in low numbers in 2009, but they became more abundant in 2010 and 24,000 cell units mL\(^{-1}\) were recorded in the outlet of Pond 47 (Figures A23, A24, A26 and A27).

- Comparison of the Euglenophyceae counts with the physicochemical parameters indicated a similar trend between the phytoplankton with COD, chlorophyll \(a\) and phaeophytin \(a\) concentrations, and turbidity (Figures A28 and A29). Lepocinclis sp., found in both Pond 47 and Pond 56, usually had counts of up to 8,000 cell units mL\(^{-1}\) (Figures A30 and A31). While it was the predominant species of Euglenophyceae in 2009, an unexplained bloom of Euglena sp. occurred in the preceding lagoon of Pond 47 in February and March 2010 (Figure A31 (a)-(b)), boosting the numbers of Euglenophyceae in the inlet and outlet samples to as high as 118,000 and 79,000 cell units mL\(^{-1}\), respectively. Such an increase in the Euglena sp. was not observed in Pond 56 at all.

5.1.5 Trends in zooplankton populations

- The zooplankton population found in the inlet and outlet of Pond 47 and 56 in 2009 and 2010, respectively, are shown in Figures A32 and A33. In 2009, Rotifera and Cladocera were more prevalent in Pond 56, whereas in Pond 47 more Copepoda were found and the presence of other zooplankton was almost negligible. The main rotifer species was Brachionus, while Asplanchna and Proalidae were detected in significant numbers on several occasions. Among the Cladocera Daphnia and Moina were the dominant species, and they were accompanied by the Copepoda (mainly Nauplii sp. and to a lesser extent, Cyclopoids). A startling difference in the zooplankton population was observed in 2010: Copepoda were dominant in Pond 47 and were present in higher numbers than in Pond 56. Nauplii sp. was regularly present, and large numbers of Cyclopoids were found occasionally. The populations of Rotifera and Cladocera were almost negligible in many of the samples.

- Since algae are one of the main sources of food for zooplankton, changes in the algal population should be reflected in the zooplankton population. The zooplankton count data were superimposed on those for algae in Figures A34 and A35), but the results indicated that there was little if any direct correlation between the total populations of phytoplankton and zooplankton.

5.2 Evaluation of the performance of the bbe Algae Online Analyser at WTP

The performance of the AOA for the quantitation of suspensions of M. aeruginosa and the green algae Chlorella sp and Euglena gracilis in HORS water separately and together was evaluated.
5.2.1 Response of the AOA to concentration of *M. aeruginosa*

The chlorophyll a (Chl-a) concentrations for *M. aeruginosa* in the concentration range of 23,000 – 94,000 cells mL\(^{-1}\) measured by the AOA on 14th January 2010 and 16th March 2011 are shown in Figure 5. There was very strong linear correlation between cell concentrations and Chl-a concentration (R\(^2\) = 0.992). The AOA readings for blue green algae in HORS water on 14th Jan 2010 and 16th Mar 2011 were 0.99 and 0 µg L\(^{-1}\), respectively. If the background blue-green algae in HORS water are taken into account, the response of the AOA to concentration of *M. aeruginosa* in both tests was fairly similar (Figure 5).

The average concentrations of the yellow substances (Y.S.) in the samples tested on 14th Jan 2010 and 16th Mar 2011 were 1.53 ± 0.15 r.u. and 1.27 ± 0.01 r.u., respectively. Yellow substances (coloured dissolved organic matter) are characterised by strong absorption in the ultraviolet and blue range which tails over into the visible region and attenuates photosynthetically active radiation. As they may interfere with the measurement of Chl-a due to the overlap of their excitation spectra with that of phytoplankton, they are measured (in terms of relative units, “r.u.”) and correction for this background absorption is made to the output of the AOA.

![Figure 5. Response of the AOA to increasing concentration of *M. aeruginosa* on 14th January 2010 and 16th March 2011.](image)

Chemical cleaning of the fluorometer occurred approximately one month prior to both tests (10th December 2009 and 15th February 2011, respectively). Therefore, deposition of material and/or development of a biofilm due to the elevated C, N and P content of the treated wastewater, could have occurred within the cuvette leading to fouling of the AOA system which may affect its performance.
5.2.2 Effect of chemical cleaning on the response of the AOA to concentration of *M. aeruginosa*

The correlation coefficients between Chl-a and cell concentration (23,000-94,000 cells mL\(^{-1}\)) before and after chemical cleaning with sodium hypochlorite (12.5% v/w) were similar (\(R^2 = 0.986\) and \(R^2 = 0.985\), respectively). However, the Chl-a readings were significantly higher after chemical cleaning (by an average of 22%) (Figure 6) and this was attributed to the removal of fouling from the cuvette. Hence fouling of the cuvette led to significantly lower Chl-a readings and so indicated that apparently lower concentrations of *M. aeruginosa* were present. Although the AOA has an automatic internal cleaning device in the cuvette, it was insufficient to prevent fouling under the conditions of use.

![Figure 6](image.png)

Figure 6. Relationship between chlorophyll \(a\) concentration and cell number for *M. aeruginosa*, before and after chemical cleaning.

5.2.3 Comparison of the results obtained from the AOA with spectrophotometric method

The Chl-a concentrations measured by the AOA and the spectrophotometric method for *M. aeruginosa* suspensions in the concentration range of 23,000-94,000 cells mL\(^{-1}\) are shown in Figure 7. At low concentrations the Chl-a values were fairly similar, however, as cell concentration increased the Chl-a concentrations measured by the AOA did not increase in the same proportion. The spectrophotometric method measures the absorbance of the Chl-a after destruction of the cells in the acetone extraction step, whereas the AOA method measures the fluorescence of the Chl-a in the intact cells in suspension. Consequently, some cells are shaded by others (shading or screening effect) with the effect increasing with cell concentration. Therefore, their Chl-a concentration cannot be determined accurately by the AOA as noted by Izydorczyk et al. (2009). The result was consistent with that obtained by Rolland et al. (2010) at the Marne Reservoir in France; they used a bbe Fluoroprobe™ *in vivo* analyser and obtained lower Chl-a values at high cell concentrations (> approx. 25,000 cells mL\(^{-1}\)) of *M. aeruginosa* compared with spectrophotometric methods.

The Y.S. concentration varied little (1.25 ± 0.05) over the concentration range for *M. aeruginosa* (green line).
The AOA and spectrophotometric methods for two green algae (Chlorella sp. and Euglena gracilis) which were prominent in the WTP effluent were compared for cell concentration ranges of 5,000-65,000 and 670-3,500 cells mL\(^{-1}\), respectively. *E. gracilis* cells are approximately 50 µm by 10 µm, and have a spiral exoskeleton (pellicle) as well as many other novel cell structures such as photosensors and endosymbiotic chloroplasts. *Chlorella* is similar in size and shape to *M. aeruginosa* (spherical and about 2-10 µm diameter). *Chlorella* sp. showed a similar trend to *M. aeruginosa* with very strong linear correlation of response with cell concentration, and a proportionately lower AOA reading with cell concentration. In contrast for *E. gracilis*, although there was a very strong linear correlation of response with cell concentration for both methods, the AOA gave a proportionately larger response with increasing cell concentration. Furthermore, unlike *M. aeruginosa* and *Chlorella* sp., for *E. gracilis* there was a significant increase in the Y.S. concentration with increasing algal concentration (from 3.2 to 6.0 r.u.). The Y.S., as well as carotenoid pigments which occur in *E. gracilis* and are known to increase the level of Chl-a fluorescence (Gruszecki et al., 1997), which would have contributed to the high apparent Chl-a levels. The difference in size (and thus less shading effect) and cell structure of *E. gracilis* compared with *Chlorella* and *M. aeruginosa* may also have played a role.

### 5.2.4 Response of the AOA to concentration of *M. aeruginosa* in the presence of *E. gracilis* or *Chlorella*

Suspensions of *M. aeruginosa* in the concentration range of 35,000-65,000 cells mL\(^{-1}\) were prepared in HORS water containing *E. gracilis* or *Chlorella* sp. with a Chl-a concentration of 5.2 µg L\(^{-1}\), measured by the AOA (equivalent to approx. 670 *E. gracilis* cells mL\(^{-1}\) or 23,000 *Chlorella* sp. cells mL\(^{-1}\)). The presence of the *Chlorella* decreased the apparent Chl-a concentration for *M. aeruginosa* by approximately 12%, while the presence of *E. gracilis* increased the apparent Chl-a concentration for *M. aeruginosa* by approximately 20% (Figure 8).
Figure 8. Effect of green algae on the response of the AOA to concentration of *M. aeruginosa*

The results demonstrate that the AOA would give an overestimate of Chl-a concentration for *M. aeruginosa* when *E. gracilis* was present, and an underestimate of Chl-a concentration for *M. aeruginosa* when *Chlorella* sp. was present in HORS water. These impacts were confirmed by the presence of *Chlorella* at 5,000-65,000 cells mL\(^{-1}\) having very little effect on readings for *M. aeruginosa* when the latter was present at either 25,000 or 50,000 cells mL\(^{-1}\). In contrast, the presence of increasing concentration of *E. gracilis* (670-3,500 cells mL\(^{-1}\)) led to large increases in apparent concentration of the *M. aeruginosa* (results for *M. aeruginosa* at 25,000 cells mL\(^{-1}\) are shown in Figure 9). There was also a large increase in Y.S. concentration for *E. gracilis*, but very little change for *Chlorella* sp.

It should be noted that the apparent cell concentration of *M. aeruginosa* decreased with the addition of *Chlorella* sp. (5,000 cells mL\(^{-1}\)) by 7.2% for *M. aeruginosa* at 25,000 cells mL\(^{-1}\) and by 4.1% for *M. aeruginosa* at 50,000 cells mL\(^{-1}\); however, it remained constant in both cases when the concentration of *Chlorella* sp. increased from 5,000 to 65,000 cells mL\(^{-1}\).
Figure 9. Relationship between the Chl-a and cell concentration for 25,000 cells mL\(^{-1}\) *M. aeruginosa* in suspensions of (a) *Chlorella* sp. and (b) *E. gracilis* in HORS water. The Chl-a concentration for *M. aeruginosa* in the absence of *Chlorella* sp. was 4.16 µg L\(^{-1}\).

5.2.5 Reproducibility of the results

Suspensions of *M. aeruginosa* (23,000 cells mL\(^{-1}\)), *Chlorella* sp. (20,000 cells mL\(^{-1}\)) and *E. gracilis* (500 cells mL\(^{-1}\)) were prepared in HORS water and the Chl-a concentration was measured in triplicate using the AOA.
after it had been chemically cleaned. The mean value, standard deviation (STD) and relative standard deviation (%STDEV) for these samples are shown in Table 3. Relative standard deviations of 10 and 14% were obtained for HORS water and HORS water containing *M. aeruginosa*, while lower values of 2% were obtained for HORS water containing *Chlorella* sp. or *E. gracilis*. The relative standard deviation for the Chl-a measured by the AOA for *M. aeruginosa* in the concentration range of 23,000 - 94,000 cells mL¹ was 10-12%. The relative standard deviation for Chl-a concentration measured by the AOA for *M. aeruginosa* slightly increased with increasing concentration of *M. aeruginosa*, however the trend was not consistent (Table 4). From Table 4, the relative standard deviation for Chl-a measured by the AOA for *M. aeruginosa* at the cell concentration range of 25,000 cells mL¹ (half of the maximum acceptable limit for recycled water at WTP) and 50,000 cells mL¹ (the maximum acceptable limit) can be estimated as 10% and 12%, respectively.

Table 3. Mean, STD and % STDEV of results obtained from the AOA (after chemical cleaning).

<table>
<thead>
<tr>
<th></th>
<th>HORS</th>
<th>HORS + <em>M. aeruginosa</em> (23,000 cells mL¹)</th>
<th>HORS + <em>Chlorella</em> (20,000 cells mL¹)</th>
<th>HORS + <em>E. gracilis</em> (500 cells mL¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>STD</td>
<td>%STDEV</td>
<td>Mean</td>
</tr>
<tr>
<td>Green (µg L⁻¹)</td>
<td>0.49</td>
<td>0.05</td>
<td>10</td>
<td>0.83</td>
</tr>
<tr>
<td>Blue-green (µg L⁻¹)</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td>3.24</td>
</tr>
<tr>
<td>Diatoms (µg L⁻¹)</td>
<td>0.06</td>
<td>0.01</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Crypt. (µg L⁻¹)</td>
<td>0.03</td>
<td>0.02</td>
<td>68</td>
<td>0.24</td>
</tr>
<tr>
<td>Y.S (r.u)</td>
<td>1.10</td>
<td>0.02</td>
<td>2</td>
<td>1.26</td>
</tr>
</tbody>
</table>

**Note:**
- Standard deviation (STD) provides a measure of variability which reflects the difference between the measurements ($x_i$) and the average measurement ($x_{AVG}$). The STD for $n$ measurements is expressed as:

$$STD = \sqrt{\frac{\sum(x_i - x_{AVG})^2}{(n-1)}}$$

- Relative standard variation (%STDEV) is the ratio of the standard variation to the average value and given as percent:

$$STDEV(\%) = \left(\frac{STD}{x_{AVG}}\right) \times 100$$

Table 4. Mean, STD and % STDEV of Chl-a concentration measured by the AOA for *M. aeruginosa* in HORS water in the concentration range of 23,000-94,000 cells mL¹ (after chemical cleaning).
### M. aeruginosa concentration (cells mL⁻¹) and Chl-a concentration measured by the AOA (µg L⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>STD</th>
<th>%STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>23,000</td>
<td>3.24</td>
<td>0.32</td>
<td>&lt;10 (9.9)</td>
</tr>
<tr>
<td>45,000</td>
<td>6.97</td>
<td>0.80</td>
<td>11</td>
</tr>
<tr>
<td>48,000</td>
<td>7.19</td>
<td>0.83</td>
<td>12</td>
</tr>
<tr>
<td>68,000</td>
<td>9.63</td>
<td>1.10</td>
<td>11</td>
</tr>
<tr>
<td>80,000</td>
<td>10.98</td>
<td>1.30</td>
<td>12</td>
</tr>
<tr>
<td>94,000</td>
<td>12.25</td>
<td>1.27</td>
<td>&gt;10 (10.4)</td>
</tr>
</tbody>
</table>

### 5.3 Validation of Abraxis Microcystin Strip Test for the detection of microcystin in the lagoon treated effluent from WTP

#### 5.3.1 Characteristics of water samples

The characteristics of the tap water, activated sludge-treated effluent, lagoon-treated effluent (collected on 30/9/09) and Yarra River water samples are shown in Table 5. The matrices varied markedly in terms of DOC, pH, conductivity, UV absorbance and SUVA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>DOC (mg L⁻¹)</th>
<th>Conductivity (µS cm⁻¹)</th>
<th>UVA</th>
<th>SUVA (mg L⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>6.15</td>
<td>2.7</td>
<td>158</td>
<td>0.034</td>
<td>1.3</td>
</tr>
<tr>
<td>Yarra River water</td>
<td>6.19</td>
<td>7.0</td>
<td>160</td>
<td>0.264</td>
<td>3.8</td>
</tr>
<tr>
<td>Activated sludge-treated effluent</td>
<td>6.40</td>
<td>10.3</td>
<td>915</td>
<td>0.333</td>
<td>3.2</td>
</tr>
<tr>
<td>Lagoon-treated water collected on 30/9/09</td>
<td>7.69</td>
<td>7.7</td>
<td>1999</td>
<td>0.160</td>
<td>2.1</td>
</tr>
<tr>
<td>Lagoon-treated water collected on 25/11/09</td>
<td>7.57</td>
<td>7.63</td>
<td>1988</td>
<td>0.228</td>
<td>2.988</td>
</tr>
</tbody>
</table>

The excitation-emission matrix (EEM) spectra of the samples are shown in Figure 10. The EEM spectra provide an indication of the total amounts of the different fluorescent classes of organic compounds within each of the water matrices. The spectra were divided into five regions: regions I and II contain peaks at shorter excitation and emission wavelengths (Ex/Em = 260/330 nm) which are related to simple aromatic proteins (Determann et al., 1994). Region III comprises peaks (Ex/Em = 260/380 nm) which are associated with fulvic acid-like materials (Nguyen et al., 2005). Region IV consists of peaks (Ex/Em = 260 – 340 nm/380 nm) which are related to soluble microbial products (SMPs) (Sheng and Wu, 2006). Region V includes peaks (Ex/Em = 260/380 nm) which are associated with humic acid-like organics (Mounier at al., 1999). The proportions and amounts of fluorescent matter in the different water matrices varied markedly, further demonstrating the differences in the characteristics of the water matrices.
5.3.2 Comparison of results measured by Abraxis strips and HPLC for different water samples spiked with microcystin-LR

The laboratory tap water, Yarra River water, lagoon-treated water and activated sludge-treated effluent described in Section 5.3.1 were spiked with microcystin-LR and the concentration of free mLRR was measured with the Abraxis strips and HPLC (Table 6). There was good correlation between the results for the Abraxis test strips and the HPLC method. In the Abraxis strip test kit with 10 ppb upper limit, there is no reference scale for the mLRR concentration of 7.5 ppb, therefore, the result for this concentration was 10 ppb.

Table 6. Comparison of free microcystin concentrations for *M. aeruginosa* as measured by the Abraxis strip test and HPLC (NT = not tested)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added mLRR</th>
<th>mLRR measured by Abraxis</th>
<th>mLRR measured by HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>10.3 mg L⁻¹</td>
<td>10 ppb</td>
<td>10 ppb</td>
</tr>
<tr>
<td>Yarra River water</td>
<td>7.0 mg L⁻¹</td>
<td>7.6 mg L⁻¹</td>
<td>7.6 mg L⁻¹</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>10.3 mg L⁻¹</td>
<td>10 ppb</td>
<td>10 ppb</td>
</tr>
<tr>
<td>Lagoon-treated</td>
<td>7.7 mg L⁻¹</td>
<td>7.7 mg L⁻¹</td>
<td>7.7 mg L⁻¹</td>
</tr>
<tr>
<td>effluent (30/09/09)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lagoon-treated</td>
<td>7.6 mg L⁻¹</td>
<td>7.6 mg L⁻¹</td>
<td>7.6 mg L⁻¹</td>
</tr>
<tr>
<td>effluent (25/11/09)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.3.3 Influence of the presence of *M. aeruginosa* on Abraxis test results

Microcystin-LR (mLR) was added to laboratory tap water, lagoon-treated water with and without the addition of *M. aeruginosa* (1.9 x 10⁷ cells mL⁻¹) and the concentrations of mLR were determined by the test strips (5 ppb upper limit) and HPLC (Table 7). There was a good correlation between the results. For the lagoon-treated water samples containing *M. aeruginosa*, an extracellular concentration of 1 ppb was apparent for the control with no added mLR, and showed as 2.5 ppb when spiked with 1 ppb of mLR, and as 5 ppb when spiked with 5 ppb of mLR (the maximum concentration which can be measured using these test strips). Lysis of the *M. aeruginosa* suspension using the QuikLyse method supplied with the Abraxis kit released 5 ppb of microcystin (measured as mLR) and so the two spiked samples also showed concentrations of 5 ppb. Further tests with other concentrations of *M. aeruginosa* in lagoon-treated water, with and without QuikLyse treatment, showed good correlation between the two methods.

Table 7. Comparison of microcystin levels in various water samples using Abraxis strip test (5 ppb limit) and HPLC (NT = not tested)

<table>
<thead>
<tr>
<th>Sample</th>
<th>0 ppb added</th>
<th>1 ppb MC-LR</th>
<th>5 ppb MC-LR added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>7.5</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Yarra River water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>7.5</td>
<td>10</td>
<td>7.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>10</td>
<td>9.5</td>
</tr>
<tr>
<td>Activated sludge-treated effluent</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>5</td>
<td>6.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>7.5</td>
<td>10</td>
<td>10.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>10</td>
<td>12.8</td>
</tr>
<tr>
<td>Lagoon-treated water (30/09/09)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.5</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>10</td>
<td>9.3</td>
</tr>
<tr>
<td>Lagoon-treated water (25/11/09)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>7.5</td>
<td>10</td>
<td>8.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>10</td>
<td>12.5</td>
</tr>
</tbody>
</table>
5.3.4 Interpretation of strip test results and the impact of sample dilution

The Abraxis strip tests provide colour comparisons for microcystin concentrations as mLR of 1, 2.5, 5 and 10 ppb. The following tests were designed to investigate whether (i) different researchers would give the same result for a given concentration, (ii) the results for intermediate concentrations could be interpolated, and (iii) whether a concentrated sample could be diluted and an appropriate reading obtained so that estimates of microcystin concentration greater than the strip test range could be obtained.

Two sets of samples containing different concentrations of mLR were prepared and the concentrations were measured using 0-10 ppb range test strips. The results were then assessed by three different researchers. For the first set of samples with mLR spiked at 2.5, 5 and 10 ppb into three different water matrices (Milli-Q water, tap water and HORQ water (collected on 11 June 10)), good agreement was obtained. The readings from the three researchers were 2.5, 5 and 10 ppb, respectively, which matched the actual mLR concentrations present.

In the second set of samples, a 15 ppb solution of mLR was prepared in tap water and then diluted to 7.5, 3.75 and 1.87 ppb. For the mLR concentration of 7.5 ppb, all three researchers reported the result as 10 ppb, significantly higher than the actual concentration. For the concentrations of 3.75 and 1.87 ppb, different readings were reported. Some felt that they could interpolate the levels, whereas others did not, and the results were reported as 4 and 2 ppb, 5 and 1 ppb, and 5 and 2 ppb. Hence, it was concluded that interpretation of the strip test results for mLR concentrations in these ranges is subjective, and tends to give an over-estimate. This would thus lead to a conservative result. Dilution of the concentrated solution with tap water led to satisfactory estimates of the concentration of microcystin in that they were high, and thus were conservative.

5.4 Investigation of the impact of sonication on algal cells

5.4.1 Sonication of *M. aeruginosa*

5.4.1.1 Immediate effect of sonication

Studying the immediate effect is a useful way of selecting potential ultrasonic densities for further examination of its long term effects. For almost all ultrasonic densities, the first 5 min of sonication gave the largest reduction in cell number (Figure 11). Joyce et al. (2010) used a much higher frequency of 580 kHz and lower power densities, but observed a similar trend. Increasing ultrasonic power and exposure time resulted in a proportionally larger reduction in cell numbers, which was consistent with general observations in sonochemistry, where an increase in ultrasonic power leads to increased sonochemical effects (Suslick, 1990; Mason et al., 1994; Zhang et al., 2006b). However, there was little difference between the percentage cell reduction after 20 minutes of sonication at both 0.186 W/mL and 0.32 W/mL, even though the power intensity was almost double.
5.4.1.2 Inhibition of growth

Higher ultrasonic densities and longer exposure time caused greater inhibition of growth (Figure 12). At 0.32 W/mL, 10, 15 and 20 min of sonication inhibited the cell growth such that the cultures did not recover to their initial cell concentration, even after a week. There was a marked inhibition of growth after 5 min of sonication at 0.32 W/mL. However, after 7 days the algal cell concentration was almost 13% of that for the untreated sample. A similar study by Zhang et al. (2006a) showed that sonication of *M. aeruginosa* at 0.32 W/mL for 5 min was so effective that after 2 weeks the cell concentration was only 14.1% that of the unsonicated sample. The results agree in that there was significant inhibition, but differed in that *M. aeruginosa* showed a faster recovery in the present work; this may be due to the use of different strains of *M. aeruginosa*. The only other ultrasonic density that showed effective inhibition of cell growth for a week after sonication was 0.186 W/mL for 20 min. For all other power settings, *M. aeruginosa* was able to recover to the initial cell concentration.

However, although greater ultrasonic power and exposure times result in greater inhibition, they lead to increased power consumption. The ultrasonic efficiency, which gives an overall indication of sonication on the organism, can be calculated by relating algal cell reduction to ultrasonic dosage (kWh m⁻³). Figure 13 shows the immediate algal cell reduction and sonication efficiency plotted against exposure time. Immediate
algal cell reduction is included in the graph since efficiency alone cannot be used as a deciding factor of best ultrasonic intensity. For example, 5 minutes of sonication at 0.043 W/mL gave the high efficiency of approximately 2.5 (kWh m$^{-3}$)$^{-1}$, but the immediate algal cell reduction was below 10%, which was extremely low. Therefore, the efficiency for a targeted cell reduction can be a useful parameter in determining the optimal operating conditions of the sonication treatment.

![Figure 13. Immediate algal cell reduction (bars) and efficiency (red line) vs. exposure time.](image)

5.4.1.3 Impact on microcystin release

Ideally, effective control of *M. aeruginosa* via sonication would involve effective growth inhibition and no or minimal release of microcystin. Sonication for 5 min at 0.043 W/mL did not induce toxin release, but 10 and 20 minutes exposure triggered a large increase in extracellular microcystin (Table 8). Five minutes of sonication at 0.32 W/mL led to an increase in the microcystin content which then decreased with increasing exposure time. These results differ from those of Zhang et al. (2006a) who showed that there was no microcystin release after 5 minutes of sonication under similar conditions, and of Ma et al. (2005) who found no microcystin in the suspension after sonication (under conditions of 0.075 W/mL, 0.15 W/mL, 0.225 W/mL for 5 minutes). The reduction in microcystin with increasing exposure can be attributed to its degradation due to sonication, which has been reported to be proportional to the power and duration of exposure (Ma et al., 2005). There was no change in microcystin concentrations after 7 days for 10, 15 and 20 minutes of ultrasonication at 0.32 W/mL, indicating effective inhibition of cell growth.

Table 8. Extracellular microcystin concentrations after sonication at 0.32 W/mL and 0.043 W/mL for different exposure times

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Microcystin concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.043 W/mL</td>
</tr>
<tr>
<td></td>
<td>0.085 W/mL</td>
</tr>
<tr>
<td></td>
<td>0.186 W/mL</td>
</tr>
<tr>
<td></td>
<td>0.32 W/mL</td>
</tr>
<tr>
<td></td>
<td>0.139 W/mL</td>
</tr>
</tbody>
</table>

*Note: The table includes columns for different exposure times and microcystin concentrations (ppb) for each condition.*
<table>
<thead>
<tr>
<th>Time after ultrasonication (hours)</th>
<th>Control</th>
<th>Sonication at 0.043 W/mL</th>
<th>Sonication at 0.32 W/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exposure Time (minutes)</td>
<td>Exposure Time (minutes)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>75±5</td>
<td>85±15</td>
<td>375±25</td>
</tr>
<tr>
<td>72</td>
<td>205±15</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>168</td>
<td>550±50</td>
<td>600</td>
<td>600</td>
</tr>
</tbody>
</table>

NT = not tested

### 5.4.3 Sonication of other algal species

Suspensions of the cyanobacterium *Anabaena circinalis* and *Chlorella* sp. were each sonicated at 0.085 W/mL for periods of 5, 10, 15 and 20 minutes.

#### 5.4.3.1 *A. circinalis*

At 0.085 W/mL, the growth inhibition of *A. circinalis* was greater than for *M. aeruginosa* for the tested exposure times (Figure 14). Conversion of the OD$_{680}$ values to proportional change in cell number for the two organisms confirmed the greater growth inhibition of *A. circinalis* after sonication. The greater inhibition for *A. circinalis* was attributed to its gas vesicles being weaker than for *M. aeruginosa* and thus prone to collapse more easily, as reported by Brookes et al. (1994).

![Figure 14. Direct comparison of effect of 5 minutes sonication for *A. circinalis* and *M. aeruginosa* at 0.085W/mL.](image)

#### 5.4.3.2 *Chlorella* sp.

Sonication at 0.085 W/mL had only minimal effect on *Chlorella*, a green alga which does not possess gas vacuoles and is of similar dimensions to *M. aeruginosa*, and was insufficient to cause reduction of cell concentration to below the initial level (Figure 15).
Figure 15. Effect of ultrasonication at 0.085 W/mL for different durations on *Chlorella* sp.

The order of decreasing growth inhibition in response to ultrasonication at 0.085 W/mL was: *A. circinalis > M. aeruginosa > Chlorella* sp. These results are in accordance with previous studies that showed selective inhibition of cells possessing gas vacuoles by sonication (Tang et al., 2004).

6. Issues Arising

- The necessary outsourcing of the sample collection and analysis for the SolarBee impact study led to issues with the timing of data receipt and hence detection of the facts that sample collection had occurred at various times during the day, and that samples were analysed some months after data collection, which may have influenced the results. Of the 45 sampling events during this project, 30 were conducted between 6 a.m. and 9 a.m., 3 were between 9 a.m. and 12 noon and the remaining 12 were conducted between 12 noon and 2 p.m. If the data for the samples collected after 9 a.m. were excluded from the analysis, it probably would not significantly impact the results for 2009 as many of these were during the colder seasons (i.e., between July and October). In contrast, their exclusion would result in a substantial loss of data during the warmer season in 2010, especially for the consecutive sampling events between 15th February and 16th March.

- Despite sharing the same supply carrier, the phytoplankton and zooplankton populations differed significantly in the inputs to Ponds 47 and 56, making their direct comparison somewhat difficult.

- As Ponds 47 and 56 are only 1.2 m deep, artificial mixing such as by the deployment of the SolarBee unit may not reduce the mean light exposure of cyanobacteria to a level that would diminish their advantage of outcompeting other phytoplankton via self-regulated buoyancy.

- The AOA can be used as an alert system for *M. aeruginosa* blooms in the HORS pond at WTP. However, the effect of the presence of green algae (such as *Chlorella* sp., *E. gracilis*) and yellow substances on the concentration of Chl-a measured by the AOA for *M. aeruginosa* (and thus potentially other cyanobacteria) should be taken into account, and the accuracy of the AOA in terms of Chl-a concentration and calibration for the presence of yellow substances should be regularly checked by other laboratory-based methods such as spectrophotometry. The installation of an automatic effective (e.g., chemical) cleaning system is recommended for maintaining consistent performance of the AOA.
7. Conclusions

The conclusions from these studies can be summarised as below.

7.1 Comparison of phytoplankton and zooplankton populations in the presence and absence of the SolarBee unit

- Similar trends for the physicochemical parameters of Ponds 47 (with SolarBee unit) and 56 were observed. Despite sharing the same supply carrier, the phytoplankton and zooplankton populations differed significantly in the inputs to the ponds, making their direct comparison somewhat difficult.

- No clear trends for cyanobacterial populations were apparent over the monitoring period. After the installation of the SolarBee unit in Pond 47 in February 2009, there was a higher incidence of increased cyanobacterial populations in Pond 56, but not Pond 47. However, the benefit of mechanical mixing to control the cyanobacterial populations was not apparent since large numbers of *Cyanophyceae* (most notably short filamentous *Planktolyngbya* sp., cf *Microcystis* and *Pseudanabaena* sp. which have significantly large cell volumes) were present in the inlet and outlet samples of Pond 47 during February and March 2010 (cf means presumed).

- The literature suggested that artificial mixing may cause a shift in the species composition in a water body. As Ponds 47 and 56 are only 1.2 m deep, and according to DO and temperature data, they show minimal stratification. Artificial mixing such as with the SolarBee unit may not reduce the mean light exposure of cyanobacteria to a level that would diminish their advantage of outcompeting other phytoplankton via self-regulated buoyancy, despite the high turbidity.

7.2 Evaluation of the Performance of the bbe Algae Online Analyser at WTP

- The AOA gave a consistent direct linear response to concentration (ranges covered 23,000-94,000 cells mL\(^{-1}\)) of *M. aeruginosa* (\(R^2\) values of at least 0.985). However, the measurement of chlorophyll \(a\) (Chl-\(a\)), and thus of the cyanobacterium, became increasingly less sensitive compared with a spectrophotometric method at cell concentrations greater than 50,000 cells mL\(^{-1}\). This was attributed to the shading or screening effect of the higher cell concentrations in the *in vivo* fluorometric method.

- The AOA cuvette was subject to fouling. Although the correlation between cell concentration and Chl-\(a\) readings remained high, lower Chl-\(a\) readings resulted indicating apparently lower cyanobacterial concentrations. This is exemplified by the loss of response (approximately 20% on average) for the concentration range of 23,000-94,000 cells mL\(^{-1}\) over one month; however the response could be restored by cleaning with 12.5% w/v sodium hypochlorite.

- The presence of the green algae *Chlorella* sp. or *Euglena gracilis* influenced the response of the AOA to concentration of *M. aeruginosa* over the range of interest at WTP (35,000-65,000 cells mL\(^{-1}\)). When *Chlorella* or *E. gracilis* at a Chl-\(a\) level of 5.2 µg L\(^{-1}\) were added to suspensions of the cyanobacterium, the presence of *Chlorella* decreased the apparent Chl-a concentration for *M. aeruginosa* by an average of approximately 12%, while the presence of *E. gracilis* increased the apparent Chl-a concentration by an average of approximately 20%. When *M. aeruginosa* was present at constant cell concentrations of 25,000 and 50,000 cells mL\(^{-1}\), in the presence of increasing concentrations of *E. gracilis* the apparent cell concentration increased, but remained the same after an initial decrease for *Chlorella*. This demonstrated that the AOA can give an overestimate or underestimate of *M. aeruginosa* populations when green algae are present in HORS water, depending on the relative numbers and species of the green algae.

- For populations of *M. aeruginosa* in the range 23,000-94,000 cells mL\(^{-1}\), the relative standard deviation for concentration of Chl-\(a\), and thus of apparent cell concentration, measured by the AOA was 10-12%.

- The results demonstrated that the AOA can be used as an alert system for cyanobacterial blooms in the HORS pond. However, the effect of the presence of some green algae on the apparent concentration of *M. aeruginosa* needs to be taken into account, the accuracy of the AOA in terms of Chl-\(a\) concentration should be cross-checked periodically with a laboratory-based method such as...
spectrophotometry, and calibration for yellow substances over an appropriate range needs to be maintained. As the deposition of particles, organisms and organics within the cuvette can negatively impact its performance, it is recommended that an automatic chemical cleaning system be installed to maintain consistent performance. Although the readings may be affected by these factors, the AOA provides real-time monitoring of algal populations and an indication of change in cyanobacterial numbers, e.g., imminent blooms. Ease of handling and relatively low maintenance costs are further advantages compared with manual cell counts which are time-consuming, and the accuracy of which is dependent on the sampling time, sample management and operator error.

7.3 Validation of the Abraxis Microcystin Strip Test for the detection of microcystin in the lagoon treated effluents from WTP

- The Abraxis test strips gave fast and reliable indication of the concentration of microcystin LR (mLR) in a wide range of water matrices, i.e., those with organic components of different types and concentrations, and with different inorganic content (as measured by conductivity).
- The presence of *M. aeruginosa* cells did not appear to interfere with the detection of mLR in the lagoon-treated effluent.
- Interpolation of mLR concentrations within the reference range provided by the manufacturer of the strips tended to be subjective and give over-estimates. Additionally, as the test strips give microcystin concentrations in terms of mLR (the most toxic microcystin congeners), the indication of toxicity tends to be high, and so conservative.

7.4 Investigation of the impact of sonication on algal cells

- Cell suspensions grown in artificial media were subjected to sonication (20 kHz) at varying power intensities (0.043-0.32 W mL⁻¹).
- Sonication led to an immediate reduction in the number of *M. aeruginosa* cells, with the initial 5 min of exposure giving the highest reduction rate. Increasing sonication power and exposure time resulted in a greater immediate reduction in cell numbers and greater inhibition of the growth of the remaining *M. aeruginosa* cells over 7 days incubation.
- Sonication for 5 min at 32 W mL⁻¹, or for a longer time (> 10 min) at a lower power intensity (0.043 W mL⁻¹) led to an immediate increase in microcystin levels in the treated suspensions. However, prolonged exposure (> 10 min) to sonication at higher power intensities reduced the microcystin concentration, which was attributed to ultrasonic degradation. Hence, the choice of suitable dosage for controlling the growth of *M. aeruginosa* should be a trade-off between the immediate and longer term sonication effects, release of toxin and power efficiency.
- Under the same sonication conditions, the order of decreasing growth inhibition for the species studied in this work was: *Anabaena circinalis* > *M. aeruginosa* > *Chlorella* sp., consistent with selective inhibition of cells possessing gas vacuoles by sonication. This demonstrates that sonication has the potential to selectively remove/deactivate cyanobacteria in wastewater treatment lagoons.
- Field scale studies should be conducted to verify the lab scale results and so provide more data for feasibility assessment and optimisation.
8. References


9. Publications and Dissemination of the Project Findings

Conference Presentations


Journal Publication

Final Report

Project Title: “Reducing and Monitoring Blue-Green Algal Blooms”

Project Number: 62M-2026

Date: March 2012
10. Appendix

Evaluation of water quality data and phytoplankton populations at Ponds 47 (with SolarBee mixer) and 56 over the period January 2009-May 2010.

10.1 Sampling Times

The sampling dates and times are summarised in Table A1.

Table A1. Date and time that samples were collected. Light shading indicates collected late morning, dark shading indicates collected after noon.

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10.2 Data for Comparison of Phytoplankton and Zooplankton Populations

Note: the complete data set has been included for ready reference (i.e., Figures 1-4 in the body of the report are also presented here.)

Figure A1. Temperature, turbidity, pH and alkalinity of Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A2. Temperature, turbidity, pH and alkalinity of Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A3. Trend for (a) dissolved oxygen and (b) temperature at different depths of Ponds 47 and 56.
Figure A4. Dissolved organic carbon (DOC) and chemical oxygen demand (COD) of Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A5. Dissolved organic carbon (DOC) and chemical oxygen demand (COD) of Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A6. Chlorophyll a and phaeophytin a analysis of Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A7. Chlorophyll a and phaeophytin a analysis of Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A8. Correlation of chemical oxygen demand (COD) with chlorophyll $a$.

Figure A9. Correlation of chlorophyll $a$ and phaeophytin $a$. 
Figure A10. Soluble and total Fe concentration for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A11. Soluble and total Fe concentration for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A12. Total algal count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A13. Total algal count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A14. Trend for soluble and total iron compared with the *Cyanophyceae* and *Aphanocapsa* sp. counts for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A15. Trend for soluble and total iron compared with the *Cyanophyceae* and *Aphanocapsa* sp. counts for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A16. *Cyanophyceae* counts for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009 (*Aphanocapsa* sp. excluded).

Figure A17. *Cyanophyceae* counts for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010 (*Aphanocapsa* sp. excluded).
Figure A18. Biovolume of various cyanobacteria found in Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet (Part 1).

Figure A19. Biovolume of various cyanobacteria found in Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet (Part 2).
Figure A20. Total algal count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009 (Cyanophyceae and Other Phytoplankton excluded).

Figure A21. Total algal count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010 (Cyanophyceae and Other Phytoplankton excluded).
Figure A22. *Coelastrum* sp. and *Pediastrum* sp. compared with Total *Chlorophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A23. *Chlorophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009 (Part 1).
Figure A24. *Chlorophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009 (Part 2).

Figure A25. *Coelastrum sp.* and *Pediastrum sp.* compared with Total *Chlorophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A26. *Chlorophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010 (Part 1).

Figure A27. *Chlorophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010 (Part 2).
Figure A28. Trend for turbidity, chemical oxygen demand (COD), chlorophyll *a* and phaeophytin *a* compared with *Euglenophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A29. Trend for turbidity, chemical oxygen demand (COD), chlorophyll *a* and phaeophytin *a* compared with *Euglenophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A30. *Euglena* sp. and *Lepocinclis* sp. compared with Total *Euglenophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A31. *Euglena* sp. and *Lepocinclis* sp. compared with Total *Euglenophyceae* count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A32. Total zooplankton count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A33. Total zooplankton count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.
Figure A34. Total algal and zooplankton count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2009.

Figure A35. Total algal and zooplankton count for Pond 47 (a) inlet and (b) outlet, and Pond 56 (c) inlet and (d) outlet in 2010.