Effect of UV Radiation on the viability of Cyanobacteria (Blue-green algae)

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ABSTRACT

Cyanobacteria can pose problems in reservoirs and source water supplies due to production of taste and odour compounds and toxins. Copper sulphate has commonly been used as an algicide to control cyanobacterial growth but due to the adverse environmental impact of copper it has been banned in some countries and has this has led to research into alternative methods. The use of UV irradiation has been suggested as one such alternative. The current study tested the effect of UV irradiation on a naturally occurring cyanobacterial population with results showing potential for its use as a control agent.

INTRODUCTION

Blooms of cyanobacteria or blue-green algae are an important issue for the water industry because of their ability to impart compounds such as tastes, odours and toxins to drinking water supplies. The problems that these micro-organisms cause will vary depending upon the season, and the characteristics of the reservoir or source water supply due to differences in climate, geology and chemistry of the water body. The two main taste and odour compounds of concern are geosmin and 2-methylisoborneol (MIB) which produce earthy-musty odours and which are a significant problem due to their low threshold of detection by humans (approximately 10 ng/L). Some cyanobacteria also produce toxins which can be generally characterised as hepatotoxins, that affect liver function, or neurotoxins that affect the nervous system. Understandably, water managers are concerned by the presence of cyanobacteria in source waters and algicides have long had a role in their control. Copper sulphate has been the algicide of choice and has generally been regarded as effective, economical and safe for operators to use, however, copper use is banned in many countries and is increasingly being regarded less favourably in others. This is due to the recognition of its adverse environmental impacts on the aquatic ecosystem and this has lead to research into alternative control methods (Burch et al, 2001). A number of alternatives are available in the market place but most have not had rigorous and scientifically valid testing.

Ultraviolet (UV) radiation is used widely in the treatment of water and wastewater treatment for destruction of a range of pathogens including bacteria, protozoa and viruses. A number of studies have indicated that the physiological processes in cyanobacteria affected by UV-B and UV-A radiation include growth, pigmentation, photosynthetic oxygen production, motility, nitrogen uptake and phycobiliprotein composition and (Hädar D, 1984; Wu *et al.*, 2005) Cyanobacteria have developed a number of mechanisms to reduce the damaging effects of UV radiation which include light driven repair of UV-damaged DNA, accumulation of detoxifying enzymes and antioxidants, and synthesis of UV protectants such as mycosporine-like amino acids and scytonemin (Ehling-Schulz and Scherer, 1999; Sinah and Hädar, 2008). The sensitivity of cyanobacteria to UV can vary within and between species (Patnaik *et al.*, 1993).

However, while cyanobacteria may have protective mechanisms against UV radiation it has been suggested that both direct and indirect exposure of cyanobacteria and algae to UV-radiation can control their growth (Bin Alam et al, 2001). Gjessing and Kallqvist 1991 showed that UV-irradiation of water containing humic substances inhibited the growth of the green algae *Selenastrum capricornutum* and exhibited a residual effect lasting several weeks. They explained that photon-initiated interactions of humic substances and other chemicals in the water resulted in the formation of oxidizing reagents such as the hydroxyl radical which provided the algicidal activity. Work in Japan with laboratory grown cultures of the common problem cyanobacterium *Microcystis aeruginosa* showed that exposure to a UV-dose of 75 mJ cm^{-2} was lethal and a smaller dose of 37 mJ cm^{-2} prevented growth for 7 days. These UV doses are within range of conventional high pressure UV-lamps used for water disinfection (~40 mJ cm^{-2}).

Bin Alam *et al* (2001) indicated that as an alternative to copper sulphate, boats equipped with UV-lamps are being used in some eutrophic lakes in Japan to control algal growth. This is potentially an attractive treatment option as it does not involve harmful chemical addition.

This study describes a preliminary investigation into the effect of artificially generated UV on a natural cyanobacterial population in Torrens Lake, South Australia, during a bloom that occurred in March 2007. The test was designed to treat the cyanobacteria in the lake water containing a natural population to account for the effects of resistance to UV radiation that wild cyanobacteria may have and any natural properties of the water that could reduce UV effectiveness.

MATERIALS AND METHODS

Water samples containing a natural mixed population of the cyanobacteria *Planktothrix mougeotii* and *Microcystis aeruginosa* were collected from the Torrens Lake in March, 2007. The test design involved the exposure of small volumes (7mL) of lake water with cyanobacteria in shallow open dishes (5cm Petri dish) to UV radiation at 256ηm using a collimated UV beam at 3 doses: 40, 80 and 120 mJ cm^{-2}. This spanned the exposure levels recommended by WHO (2004) to remove bacteria (7 mJ cm^{-2}), viruses (59 mJ cm^{-2}) and protozoa (10 mJ cm^{-2}) from drinking water. The collimated beam apparatus housed a low pressure UV (LP-UV) lamp which was calibrated using an IL1400 (UV Process Supply Inc.) radiometer. The UV light entered the suspension with a near zero degree angle of incidence and was homogeneous across the surface area. UV dose delivered to the suspension was calculated using measurements of incident UV intensity, exposure time, suspension depth, and the absorption coefficient of the suspension. The absorption co-efficient of Torrens Lake water was determined using a UV-Vis spectrophotometer to be 0.692 cm^{-1} at 254ηm. The suspension was slowly mixed using a magnetic stirrer and bar to ensure uniformity of exposure to UV. To achieve exposure doses of 40, 80 and 120 mJ cm^{-2} the lake water samples were exposed for 7 min 11 s, 14 min 22 s and 21 min 32 s respectively.

The viability of cyanobacteria was determined 24 hours after UV exposure by a technique involving microscopic examination and cell counting and the use of activity stains which give an indication of cell damage and viability. The stains used were Fluorescein diacetate (FDA) and Propidium iodide (PI). FDA passes through cell membranes and is hydrolysed by intracellular esterases of healthy cells to produce fluorescein (a fluorescent product) which exhibits a green fluorescence when excited with blue light. PI only passes through the membranes of dead or dying cells and stains DNA in the cells an orange colour. "Total Cell" number was measured using a Sedgewick-Rafter counting chamber and microscope. The proportion of viable cells expressed as a percentage was measured after treatment with vital stains using a Lund Cell counting chamber and fluorescent microscopy. "Viable Cell" number was then determined using percentage of living cells and the "Total Cell" number.

Each exposure was performed in triplicate. Controls were included which consisted of lake water samples held in experimental set-up (stirred in dishes) for the same time periods as the exposed cell suspension but without exposure to UV. A single control was included for each dosage and an average determined from the combined value. This removes any effect from variables other than UV exposure on cell numbers and viability.

After exposure, the cell suspensions were transferred to 50mL centrifuge tubes and incubated at 25oC in a controlled environment cabinet under artificial illumination of 30 µmol photons $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) with light/dark cycle of 12h:12h for a period of 24 hours to reproduce conditions similar to that experienced in the environment.

ANOVA was used to determine a significant ($p<0.05$) difference between total cell numbers and total viable cell numbers (STATISTICA®).

RESULTS AND DISCUSSION

Figure 1 shows the effect of 40, 80 and 120 mJ cm^{-2} of UV radiation at 254ηm compared to the control on total cell number and viable cell number of *P. mougeotii*, and *M. aeruginosa* 24 hours after exposure.

There was no significant ($p<0.05$) decrease in the total number of *M. aeruginosa* cells 24 hours after cell suspensions were exposed to UV radiation at all three doses compared to the control (Figure 1A). All the cells present were viable after exposure to 40 and 80 mJ cm^{-2} i.e. there was no significant difference between total cell numbers and total viable cell numbers ($p>0.05$). However, at the highest irradiance of 120 mJ cm^{-2} the number of viable cells for *M. aeruginosa* was significantly lower than the control and the other two UV exposures ($p<0.05$).

The viability of *P. mougeotii* was extremely low i.e. approximately 10 % of the total cells present were shown to be viable at all three exposures (Figure 1B). It would appear that exposure to UV radiation at the doses tested made the cell membrane leaky which allowed propidium iodide to penetrate and stain the DNA and so indicate that the cell

was dead. However, the cell membrane still retained enough integrity to preserve its shape, and so appeared as a whole cell in the total cell count. Cell numbers would be expected to decrease over time as the membrane starts to breakdown.

Results suggest that *M. aeruginosa* was more resistant to the negative effects of UV than *P mougeotii.*

CONCLUSIONS

The results of this preliminary trial have shown that exposure to UV radiation at 254ηm has a significant effect on the viability of natural populations of cyanobacteria but appears to vary between cyanobacterial types. For example, the viability of *M. aeruginosa* cells was still high even after exposure at 120 mJ cm^{-2} compared to cells of *P. mougeotii* which showed a significant reduction in viability at an exposure of 40 mJ cm^{-2}. This is not surprising as *M. aeruginosa* (**Figure 2**) form large spherical colonies where cells located in the middle would be protected from incident radiation by surrounding cells. *P. mougeotii* are free-floating long chain filamentous cyanobacteria (**Figure 3**) making them an easy target for UV radiation. The current study has shown that the effect of UV radiation and its use as a cyanobacterial control method warrants further investigation.

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Figure 1: The effect of UV on the viability of natural populations of cyanobacteria 24 hours after exposure. The figure shows numbers of total and viable cells of **A**; *Microcystis aeruginosa*, and **B**; *Planktothrix mougeotii*, in a Torrens Lake water sample 24 hours after exposure to UV irradiation (254nm) at doses of 0, 40, 80 and 120 mJ cm^{-2}

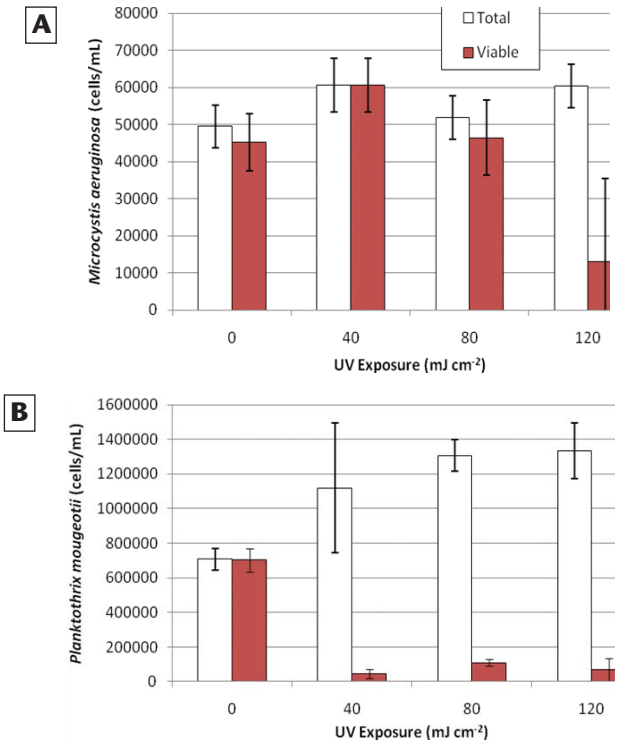

Figure 2: Colony of *Microcystis aeruginosa* from Torrens Lake.

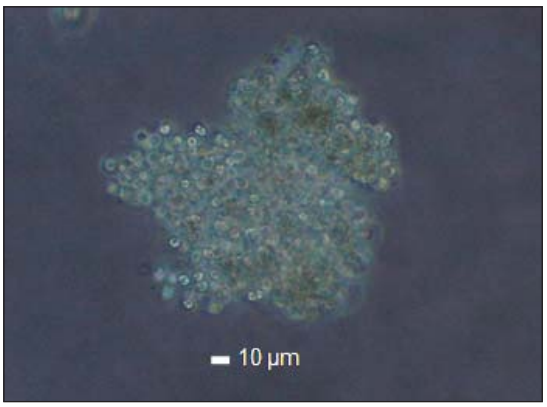

Figure 3: Filaments of *Planktothrix mougeotii* from Torrens Lake.