Integrated membrane systems incorporating coagulation, activated carbon and ultrafiltration for the removal of toxic cyanobacterial metabolites from *Anabaena circinalis*


**ABSTRACT**

The use of integrated membrane systems (a train of treatment processes incorporating one or more membranes) is increasing globally as the technology is very effective for the production of high quality drinking water. In this investigation a laboratory scale integrated membrane system (IMS) featuring coagulation, powdered activated carbon (PAC) and ultrafiltration (UF) was investigated for the removal of an Australian strain of the cyanobacteria *Anabaena circinalis* and the cyanotoxin it produced. Three coagulants were compared, aluminium chlorohydrate (ACH), aluminium sulphate (alum) and an engineered aluminium coagulant referred to as high performance aluminium chlorohydrate (HPAC). PAC (Acticarb PS1000) was tested to determine adsorption of extracellular saxitoxin. Removal of *A. circinalis* cells was 100% by UF alone and the removal of cells prior to the membrane by coagulation reduced fouling attributed to algogenic organic material. Alum was the least efficient coagulant for removal of cells while ACH and HPAC were similar. Saxitoxin removal reached a maximum of 80% using ACH and PAC. The UF-IMS was challenged using a natural bloom of *A. circinalis* that occurred in the Myponga Reservoir in South Australia.

**Key words** | *Anabaena circinalis*, coagulation, cyanobacteria, powdered activated carbon (PAC), ultrafiltration

**INTRODUCTION**

Cyanobacteria (blue-green algae) are a major problem for the water industry as they can produce metabolites toxic to humans in addition to taste and odour (T&O) compounds that make drinking water aesthetically displeasing (Carmichael 1992, 1994; Chorus & Bartram 1999; Fleming et al. 2002). It is likely that this problem will be intensified by the effects of climate change through reservoir warming (Paerl & Huisman 2008; Paul 2008; Davis et al. 2009). Tropical cyanobacterial species are also becoming more prevalent at more temperate climates (Falconer 2005). The effective removal of cyanobacterial metabolites is therefore an increasingly important research priority for the worldwide water industry.

Treating cyanobacteria in reservoirs using algicides can lead to cell lysis (Chow et al. 1999) which, in turn, can lead to higher concentrations of extracellular metabolites which are difficult to remove by conventional flocculation and filtration (Drikas et al. 2001; Bruce et al. 2002). In the absence of any damage to the cells, conventional treatment can be effective for the removal of the intact cells and therefore the majority of the compounds, for example, microcystin can be up to 98% intracellular (Chow et al. 1997). With regards to extracellular metabolites, powdered activated carbon (PAC) has been shown to be an effective method for removal of toxins and T&O compounds, including 2-methylisoborenol (MIB) and geosmin (GSM) (Newcombe 2006). As membrane technology has become more economically viable, the demand for information on the application of membranes for cyanobacterial metabolite removal has increased. In addition to this,
processes such as coagulation and PAC dosing are now being integrated with membrane processes such as ultrafiltration (UF) and microfiltration (MF) for particulate removal. UF as a single treatment process has previously been investigated in several studies. In a study by Chow et al. (1997) special flat-sheet MF and UF membranes were assessed for their ability in cross-flow and dead-end operation to remove the cells and toxins of a *Microcystis aeruginosa* bloom. The membranes removed >98% of the cells, as the pore size of both membranes was an order of magnitude smaller than the size of the *M. aeruginosa* cells. In another series of UF experiments, with a cell-culture of *Planktothrix agardhii* (Gijsbertsen-Abrahamse et al. 2006), a small concentration of intracellular microcystin was released and transferred to the permeate. The study showed that release of up to 2% of the intracellular microcystin is caused by shear due to the filtration process itself. Campinas & Rosa (2002) investigated the use of UF for the removal of *M. aeruginosa* however this study maintained a focus on cell lysis during the UF process and the effect that ageing has on this lysis. While cell lysis occurred for each cell age, microcystin concentration was always identical or lower in the permeate than in the feed water indicating some adsorption of the toxin by the membrane.

Integrated membrane systems (IMS) may be more effective for the removal of cyanobacterial metabolites. Mouchet & Bonnélye (1998) suggested that a PAC and UF system, with PAC applied either alone or following conventional sedimentation and/or ozonation, was the most promising system for simultaneous removal of virtually all cyanobacteria and their toxins. Lee & Walker (2006) investigated the efficiency of a PAC-UF process to remove microcystin-LR (MCLR). Compared with PAC adsorption alone and UF alone, the combined PAC-UF system had a higher removal efficiency for both the polyethersulfone (PES) and cellulose acetate membranes. However, the removal profiles differed for the two membranes. UF using cellulose acetate membranes without PAC addition did not remove MCLR while the PAC-UF system resulted in 70% removal of the toxin. The removal of MCLR by the PAC-UF system as a function of time followed a trend similar to that of PAC adsorption alone, which suggests that PAC adsorption was the dominant removal mechanism. In the case of the PES membrane, the PAC-UF system removed MCLR more effectively than membrane filtration or PAC adsorption alone, since both PAC and the PES membrane adsorbed MCLR. Campinas & Rosa (2006b) also investigated the use of PAC-UF for cyanobacterial metabolite removal. In the absence of background natural organic material (NOM), PAC-UF with 10 mg/L PAC and up to 20 ug/L MCLR equivalents feed concentration, achieved 93–98% removal. While 10 mg/L PAC effectively controlled about 5 ug/L MCLR equivalents in a model water with 2.5 mg/L NOM or with *M. aeruginosa* culture, a PAC dose of 15 mg/L was unable to achieve effective removal with a water containing higher concentrations of NOM (5 mg/L) and microcystin (20 ug/L MCLR equivalents).

Most studies have investigated coagulation and UF or PAC and UF; however no studies to date have investigated the combination of all three processes. Coagulation prior to UF has become a common practice since the late 1990s (Howe & Clarke 2002) and has the potential to remove NOM, which has been recognised as the major foulant to UF (Jucker & Clark 1994; Clark & Lucas 1998; Combe et al. 1999; Jones & O’Melia 2000; Aoustin et al. 2001). In particular, algogenic organic material (AOM) contains a fraction of polysaccharides and proteins which has been shown to form an adhesive fouling layer on membranes which further contributes to membrane fouling (Lee et al. 2000; Fan et al. 2001; Her et al. 2004). While PAC has the potential to remove extracellular toxin and T&O, it also has the ability to remove NOM (Newcombe 2006) which can further prevent membrane fouling. Consequently, an IMS incorporating coagulation and PAC may improve both removal of cyanobacterial metabolites and membrane fouling by AOM.

To date no studies have investigated the removal of *Anabaena circinalis* in an IMS or the removal of saxitoxins by membranes. This study is warranted as the occurrence of toxic *Anabaena* blooms are frequent and occur globally (Baker 1991). Furthermore, there is a lack of studies which have investigated the use of polyvinylidene fluoride (PVDF) UF membranes which are commonly used in full scale membrane plants due to their high resistance to chlorine and cleaning chemicals. The aim of this study was to investigate the use of an IMS system incorporating coagulation, PAC and UF for the removal of intracellular and extracellular saxitoxin from *A. circinalis* and the impact of these treatments on the UF flux decline through preventing NOM fouling on the UF membrane. This is the first IMS study to investigate the removal of cultured cyanobacterial blooms to establish removal of both intracellular and extracellular cyanobacterial metabolites.

**METHODS**

**Cell culture**

A laboratory strain of *A. circinalis* (ANA 188B), known to produce saxitoxins, was grown in artificial seawater medium
(ASM-1) (Gorham et al. 1964) at 25°C under continuous illumination. Cultures were harvested at the late exponential phase of growth, which corresponded to 14 d after inoculation.

**UF-IMS laboratory testing**

To determine the effectiveness of coagulation and PAC dosing within an IMS, an UF laboratory trial was conducted. A testing schedule was devised to study the removal *A. circinalis* with different coagulants in equivalent concentrations of Al³⁺ with and without PAC. Raw water used was collected from the Mannum-Adelaide pipeline at Palmer water treatment plant (WTP). Water quality parameters of the raw water used were dissolved organic carbon (DOC) 4.0–6.2 mg/L, UV₂₅₄ absorbance (UVabs) 0.060 to 0.065 cm⁻¹ and turbidity 12–15 NTU. Cultured *A. circinalis* cells were dosed at 100,000 cells/mL into the feedwater. To replicate the conditions at Palmer WTP, the UF experiments utilised a flocculation time of 9 min at a slow stir speed of 20 rpm and a detention time of 11 min to simulate the membrane tank. Three coagulants were trialled at 2.2 mg/L of Al³⁺: ACH, aluminium sulphate (alum) as Al₂(SO₄)₃.18H₂O and high performance aluminium chlorohydrate (HPAC), which was developed by the Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences. The coagulant is a tailored formulation with high Al₁₃ polymer speciation, designed for improved AOM removal. The PAC used was Acticarb PS1000 which is a steam activated coal based carbon.

A laboratory scale UF unit (Figure 1) was used which utilised hollow fibre PVDF membranes with a 0.02 µm pore size. Ten 10 cm UF fibres were potted using epoxy resin and compacted using ultra pure water. Membranes were operated in an outside-in configuration at a pressure of 160 kPa. Membrane integrity was established using turbidity removal. Each experiment showed removal of turbidity to 0.1 NTU from raw water values of 12–15 NTU. Each experiment consisted of four operation periods. The first was a ultrapure water flush, the second using only the feedwater (Palmer water dosed with *A. circinalis* cells), the third using coagulant dosing and the final using both coagulant and PAC at 20 mg/L. Coagulant and PAC were dosed into a flocculation tank agitated at 20 rpm with a detention time of 9 mins. A membrane tank prior to the membrane housing ensured a total floc growth time of 11 mins, similar to the Palmer WTP as mentioned above. Between each operation period a 2 min backwash involving air scouring and ultrapure water was performed. After each experiment the membrane was cleaned using two protocols: 1) citric acid at pH 2 and 2) NaOH at pH 10. The same membrane was used for each experiment.

**Analysis**

Samples analysed for saxitoxin were undertaken via enzyme linked immunosorbent assay (ELISA) purchased from Abraxis LLC (USA). Samples for analyses were diluted in order to bring the samples within the working range of the assay (1:20). These analyses were carried out according the manufacturer’s protocol. The Abraxis ELISA is an antibody-based assay and cross-reactivities for the following saxitoxin analogues are: <0.2% GTX1&4, 1.3% for NEO, 23% for GTX2&3, 29% dcSTX and 100% for STX, as stated by the manufacturer. The lower limit of detection and coefficient of variation for the Abraxis ELISA assays were 0.02 ppb and <15%, respectively. Conversion factors (Oshima 1995) were used to express the toxicity of the sum of the variants as

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*Figure 1 | Process diagram of the Ultrafiltration-Integrated Membrane System.*
STX-equivalents (STX-eq) owing to the differing toxicities and concentrations of the individual saxitoxin variants. 

*A. circinalis* cells were enumerated on a compound microscope in a Sedgewick–Rafter counting chamber after preservation in Lugol's iodine. Cell counts were carried out to a minimum precision of 30%.

A Hach ratio turbidimeter (model 2100AN) was used to give a direct reading of the turbidity of a sample in NTU. Samples for DOC and UVabs were filtered through 0.45 μm pre-rinsed membranes. UVabs was measured at 254 nm through a 1cm quartz cell. DOC was measured using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA).

## RESULTS AND DISCUSSION

### Cell removal

UF completely removed the *A. circinalis* cells regardless of whether coagulant or PAC was used due to the UF's pore size being an order of magnitude smaller than the cells, which was also observed by *Chow et al.* (1997) (results not shown). The coagulation process removed 95% of the cells when using HPAC and ACH (Figure 2). When using alum the removal was 65%. The coagulation and PAC process made no further impact on removal of *A. circinalis* cells when using HPAC and ACH. The use of PAC in conjunction with alum improved removal to 85% compared with using alum alone. The addition of PAC during alum coagulation may have enhanced flocculation of the *A. circinalis* cells and subsequently improved removal, a phenomenon which has been implied by *Cook et al.* (2001).

### Saxitoxin removal

Total saxitoxin (intracellular and extracellular components) concentration was determined to be 2.2–2.7 μg/L STX-eq in the feed water to the UF membrane of which 31–38% was extracellular (0.7–0.8 μg/L STX-eq). Results show that when using coagulant or coagulant with PAC, up to 78% removal of total saxitoxin was achieved in the membrane tank as intact *A. circinalis* cells were removed via this process prior to contact with the membrane (Figure 3). The coagulation process removed intracellular saxitoxin while PAC removed extracellular saxitoxin. During the coagulation process, with or without PAC, alum was less effective than ACH or HPAC for total saxitoxin removal.

During the combined IMS process incorporating coagulation, PAC and UF membrane filtration, it appeared that PAC adsorption was the major mechanism for the removal of extracellular saxitoxin as no further removal was observed by the membranes. This was evident when using HPAC and alum as the coagulants during the IMS process. However, when using ACH as the coagulant during the IMS process an improvement in extracellular saxitoxin removal was observed (Figure 4). The ACH experiment was the first in the series that was conducted and was carried out on a virgin membrane. It is likely that there was a larger component of extracellular saxitoxin adsorption to the membrane fibres before the fibres became saturated with saxitoxin. This is consistent with other previous studies (*Chow et al.* 1997; *Gijsbertsen-Abrahamse et al.* 2006; *Campinas & Rosa* 2010b).
In this study lower percentage removals of extracellular toxins were observed compared with previous studies (Newcombe & Nicholson 2004; Ho et al. 2009). It is postulated that the coagulation process would reduce the effectiveness of PAC adsorption due to entrapment of PAC particles within the floc structure which could possibly reduce the kinetics of toxin adsorption (Cook et al. 2001; Ho & Newcombe 2005).

Flux decline

The decline in specific flux of the UF alone when fed with raw water dosed with *A. circinalis* cells is shown in Figure 5 (T = 0–120 min). The addition of coagulants reduced flux decline (T = 120–190). When PAC was used in conjunction with the coagulants (T = 190–270 min), HPAC further reduced flux decline while the combinations of alum/PAC and ACH/PAC showed little difference from using the coagulants alone.

The reduction in flux decline when using coagulants may be attributed to the removal of NOM prior to filtration by the membrane as NOM has been shown to be a membrane foulant (Jucker & Clark 1994; Clark & Lucas 1998; Combe et al. 1999; Jones & O’Melia 2000; Aoustin et al. 2001). The feed water DOC was 4.0–6.2 mg/L and removal of DOC using only the membrane was 29–41%. When using coagulation, DOC removal was 40–55% for ACH and HPAC. The coagulation process removed less DOC when using alum (20%) (Figure 6). During the coagulation process, removal of UVabs (Figure 7) was similar to DOC removal with removal for HPAC and ACH. Alum gave better UVabs removal than DOC removal. Addition of PAC improved removal of DOC to between 5 and 21% for ACH and HPAC while alum showed negligible improvement. PAC improved UVabs removal by a further 10–18% for each coagulant.

As mentioned above, when using coagulant in an IMS process, removal of *A. circinalis* cells was achieved by the coagulation process alone. This has the potential to minimise the fouling on the UF as observed in Figure 5 through a reduction in flux decline when using coagulants. As coagulation removes NOM, this may improve the longevity of the process.
membrane by preventing irreversible NOM fouling (Dixon et al. 2010). In addition, cyanobacterial cells contain polysaccharides and proteins that can form an adhesive layer on the membrane surface which further contributes to fouling (Lee et al. 2000; Fan et al. 2001; Her et al. 2004).

**SUMMARY AND CONCLUSIONS**

An IMS (incorporating coagulation, PAC and UF) was investigated for the removal of *A. circinalis* and intracellular and extracellular saxitoxin. The impact of these treatments on the UF flux decline was also studied. The major findings of this study were:

- *A. circinalis* cells were completely removed using UF alone; however, when coagulation and PAC were incorporated, cells were removed by this process. Alum was the least effective coagulant for this purpose.
- Total saxitoxin removal by the UF alone was 44–65% and addition of coagulant made little improvement.
- UF fouling by NOM was diminished using coagulant and coagulant with PAC, as demonstrated by reduced flux declines. HPAC with PAC was the superior method for mitigation of flux decline.

The application of IMS is warranted as cyanobacteria can compromise water quality and treatment in many ways. IMS can provide a multibarrier treatment approach which is favoured in risk-based approaches including many global water safety plans.

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