Odourous algal-derived alkenes: differences in stability and treatment responses in drinking water

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Abstract Drinking water supplies are often impacted by taste and odour (T/O) episodes caused by algal volatile organic compounds (AVOCs) from algal blooms. Treatment and control of these events is important to utility operators, as customer confidence in the safety of public drinking water supplies is based primarily on their palatability and odour. To manage T/O outbreaks successfully, knowledge about treatment responses of AVOCs and anticipation of their outbreaks are thus of major importance to the water industry. The Glenmore Reservoir and water treatment plant (GWTP) supplies drinking water to over 50% of the ca. 1 million consumers in Calgary (Alberta). Despite low nutrients and high raw water quality, the reservoir experiences periodic outbreaks of fishy/floral T/O, caused by chrysophytes and diatoms (Uroglena americana, Dinobryon spp., Synura petersenii, Asterionella formosa). These odours are produced by the unsaturated C7–C10 alkenes 2,4-heptadienal, 2,4,7-octatriene, 2,4-decadienal and 2,4,7-decatrienal, generated during from the enzymatic breakdown of algal polyunsaturated fatty acids (PUFAs). The formation, persistence and stability of these compounds in both the raw water and treatment plant is not well understood.

Keywords Aldehydes; drinking water treatment; PUFA; SPME; taste and odour

Introduction

The palatability of drinking water in terms of its clarity, taste and odour (T/O) is a consumer’s only direct indication of water quality (Montiel, 1983; McGuire, 1995; Ridal et al., 1999; Satchwill et al., 2001). For this reason, both suppliers and consumers place great emphasis on these organoleptic properties of drinking water (Montiel, 1983; Persson, 1983, 1992; Skulberg, 1988; Watson et al., 1999, 2000a, b). For surface water supplies, the primary water treatment processes of chlorine disinfection and coagulant assisted filtration generally yield water of good clarity and free of pathogenic organisms (Montiel, 1983). Treatment and removal of T/O compounds can be more difficult.

Identification, quantitation and treatment of these AVOC are important to the water treatment industry (Watson et al., 1999, 2000a, b). Specific knowledge of the sources and composition of T/O compounds is needed for utility operators to maintain public confidence in the safety of municipal drinking water supplies. Drinking water T/O problems are extensive and pervasive (Persson, 1983). T/O complaints are reported on a global scale, and they account for the largest single class of consumer complaints to water utilities (McCallum et al., 1998).

Both geosmin and 2-MIB are potent T/O agents, and these earthy-musty odour compounds are responsible for the majority of reported T/O events in surface waters. Geosmin

1 The opinions expressed are those of the authors and do not represent those of Health Canada

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and 2-MIB are produced throughout the cell’s lifecycle, can accumulate within the cell and then be released into the environment either through cell leakage or cell lysis. Importantly, these compounds are environmentally stable and can undergo long range transport, affecting areas distant from where the compounds are produced (Ridal et al., 1999; Satchwill et al., 2001). There are natural mechanisms for the breakdown of these compounds, however, these processes are slow relative to transport and production.

The occurrence of geosmin and 2-MIB is common in highly productive aquatic systems that support cyanobacteria. In less productive systems that are nutrient limited, such as the Glenmore Reservoir, other species may dominate. Chrysophyceae algae such as Uroglena spp. and Dinobryon spp. are known to cause fishy odours from the breakdown of polyunsaturated fatty acid primary metabolites to unsaturated aldehydes by enzymatic activity (Yano et al., 1988; Watson et al., 1999). Synura petersenii is associated with E2,Z6nonadial, which has a cucumber odor and an odour threshold concentration (OTC) of 80 ng·L⁻¹ (Wee et al., 1994). Other odorous aldehydes such as E2,E4-heptadienal, E2,E4-decadienal and E2,E4,Z7-decatrienal are produced by a variety of species, including Dinobryon, Uroglena, Asterionella (Jüttner, 1995; Watson et al., 1999). In the Glenmore Reservoir Uroglena are associated primarily with the production of heptadienal, while decadienal and decatrienal are associated with Dinobryon. These conjugated aldehydes have a fishy–cod liver oil odour, and the estimated OTCs range from 1 to 50 μg·L⁻¹ (Ke et al., 1975; Watson et al., 1999). The production of 1,3,5-octatriene is associated with the diatom Asterionella. Unlike geosmin and 2-MIB, these odorous alkenes are produced only when the cell breaks down and releases its contents, which includes PUFAs and lipoxigenases (Cotsaris et al., 1995). Knowledge of the T/O chemicals present and the source species is essential for effective control or prevention of T/O episodes (Persson, 1992). As the odorous compounds are stored intracellularly as fatty acid precursors, it may be possible to prevent formation of T/O compounds by elimination of the intact algae.

The unsaturated AVOC that are formed are subject to isomerisation of the conjugated 2,4-diene moiety from the E,Z to an equilibrium mixture of E,E and E,Z forms (Watson et al., 1999, 2000a; Satchwill et al., 2001). These geometric isomers have distinct physical and physiological properties. Chromatographic analyses will resolve the different isomers, therefore each retention time needs to be monitored to ensure an accurate estimation of the total mass is made. The 2,4-E,Z isomers of the C10 aldehydes are characterised by rancid/fishy odours, while the 2,4-E,E isomers have less objectionable and less potent cucumber/floral odours.

In a previous study, the use of aluminum sulphate (alum) coagulation combined with conventional gravity sedimentation or dissolved air flotation was demonstrated to be effective at removing algal biomass from natural waters (Satchwill, 2001; Satchwill et al., 2001). Importantly, these water treatment processes did not impair the integrity of the algal cells, and were therefore also effective in removing the AVOC. In this study, the effects of additional water treatment processes in use at the Glenmore Water Treatment Plant (GWTP) were evaluated, namely in-plant storage, chlorine oxidation and alum coagulation. These conventional processes were applied to cultures containing AVOC producing algae that have been responsible for T/O events in the Glenmore Reservoir. The treatment response and change in production of AVOC that resulted from each process was measured.

Experimental methods

Headspace SPME

In this study, we used headspace phase solid phase microextraction of headspace (H-SPME) with gas chromatography-mass spectrometry (GC-MS) to analyse natural waters and algal culture material. Headspace sampling was selected in order to minimise
matrix effects and fouling of the SPME fibre. This method was able to detect alkenes and other T/O compounds in both ambient waters and cultures at ng·L$^{-1}$ levels. The limit of quantitation ranged from <10 to 500 ng·L$^{-1}$ over the entire group of compounds measured.

Optimisation of this method and its application to the analysis of T/O compounds has been reported previously (Watson et al., 2000a). Briefly, 30 mL samples were salted out with 6 g NaCl and placed with a stir bar in 43 mL septum vials. An internal standard solution of biphenyl-d$_{10}$ was added, the vial was sealed with Teflon$^\text{®}$ lined cap and then gently agitated to dissolve the salt. The vial was placed in a stirrer equipped heater block preheated to 65 $^\circ$C. The aqueous phase was stirred to vortex and the headspace phase was sampled for 30 min with a polydimethylsilicone/divinyl benzene SPME fiber. Biological samples such as ambient waters and culture material were prepared immediately prior to analysis to minimise sample degradation.

The addition of NaCl is used in the analytical protocol to improve the SPME process by partitioning dissolved T/O compounds into the headspace phase. More importantly, the osmotic shock caused by salt addition to biological samples releases cellular metabolites and is essential to the formation of AVOCs.

**GC-MS**
Following extraction, the SPME fibre was immediately desorbed for 60 s at 250 $^\circ$C onto a 6890/5972 Agilent GC-MSD, operated in splitless mode and equipped with a DB-1701 fused silica capillary column. The mass range of 40–300 m/z was scanned, rather than operating in selected ion-monitoring mode. This facilitated peak identification, which was complicated due to the low m/z of the ion fragments and the formation of geometric isomers. In addition, broader chemical surveys could be performed for other compounds.

**Method precision and accuracy**
SPME and headspace extraction methods are equilibrium sampling processes, and complete recovery of all analytes from the sample phase is not possible, such as can be achieved by liquid or solid phase extraction. For the T/O compounds under study it was found that the absolute recovery by H-SPME ranged from 1.9% for heptadienal to 13% for decadienal, relative to the total mass of analyte in the sample (Satchwill, 2001). However, SPME is very efficient in that 100% of the sample recovered is injected onto the analytical instrument. Method precision and quantitation were demonstrated over the range of 0.5–10 $\mu$g·L$^{-1}$. Quantitative method detection limits are presented in Table 1 and were calculated using the procedure outlined by *Standard Methods* (APHA et al., 1998) from nine replicate 1 $\mu$g·L$^{-1}$ standards.

The H-SPME procedure was validated in an inter-laboratory split sample study (Watson et al., 2000a). Two laboratories analysed five replicates of each of two dissimilar samples. The results were evaluated with a two-way analysis of variance. There were no statistical differences in the results obtained by the two laboratories ($p < 0.0001$). The excellent

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method detection limits ($\mu$g·L$^{-1}$)</th>
<th>Standard deviation ($\mu$g·L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptadienal</td>
<td>0.51</td>
<td>0.18</td>
</tr>
<tr>
<td>Nonadienal</td>
<td>0.62</td>
<td>0.22</td>
</tr>
<tr>
<td>Decadienal</td>
<td>0.51</td>
<td>0.18</td>
</tr>
<tr>
<td>Decatrienal</td>
<td>0.54</td>
<td>0.12</td>
</tr>
</tbody>
</table>
agreement in results between the two laboratories demonstrates the repeatability of the H-SPME procedure.

Evaluation of storage conditions on sample stability

It has previously been demonstrated that terpenoid T/O compounds in ambient surface water are sufficiently stable to allow recovery after 3 weeks of storage under simple conditions (i.e. refrigerated; dark), however the alkene compounds are less robust (Palmentier et al., 1998; Satchwill, 2001). The alkene AVOCs are produced by the enzymatic breakdown of cellular PUFAs, which are in turn susceptible to further oxidation. The stabilities of synthetic standards were evaluated in three different matrices while stored refrigerated and protected from light. Chemical standards containing both terpenoid and alkene T/O compounds were prepared in abiotic reagent water, a biological matrix of high organic content prepared from algal culture material diluted with ambient reservoir water, and the biological matrix with 20% (w/v) salt added. Samples were stored for 48 and 336 h. The relative change in alkene AVOC concentrations from algal cultures stored for 5 h with and without salt addition was also examined.

Evaluation of in-plant processes on mixed algal cultures

A mixed assemblage was prepared from cultures of Asterionella formosa, Mallomonas, Synura, Uroglena americana and Dinobryon divergens. This algal stock solution was then used to assess the effect of in-plant storage, chlorine oxidation and alum coagulation were investigated in a series of experiments, as presented in Table 2.

The sample stability data generated from the above experiments demonstrated that control of storage and treatment time is essential. Therefore, a careful sample and analysis schedule was developed to minimise artifacts which may arise from temporal variations. All analyses were completed within 24 h of preparing the algal stock solution to minimise variations that may arise from population and life phase changes. Samples were stored without headspace in darkened conditions at 4 °C.

A treatment time of 5 h was selected based on the hydraulic capacity of the GWTP, and the chemical application rates selected were again representative of those in the full scale GWTP. The experimental treatments consisted of: (1) in-plant storage with no additional treatment, (2) addition of 1.8 mg·L⁻¹ chlorine as an oxidant; (3) addition of 16 mg·L⁻¹ alum as a coagulant, and (4) the combined addition of chlorine and alum at the same concentration. Two additional groups of samples were analysed. One was subject to no treatment or storage, and was used as an experimental control group. The second group was subject to the addition of 5% (w/v) NaCl, which was sufficient to induce cell

Table 2 Treatment conditions evaluated

<table>
<thead>
<tr>
<th>Treatment code</th>
<th>Storage time (h)</th>
<th>Salt (%w/v)</th>
<th>Chlorine (mg·L⁻¹)</th>
<th>Alum (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 h</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NaCl</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cl₂</td>
<td>5</td>
<td>0</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Cl₂/Alum</td>
<td>5</td>
<td>0</td>
<td>1.8</td>
<td>16</td>
</tr>
<tr>
<td>Alum</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Cl₂</td>
<td>5</td>
<td>0</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Cl₂/Alum</td>
<td>5</td>
<td>0</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Alum</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

NT = control sample with no treatment. All other samples received 5 h contact time. 5 h = stored without chemical treatment; NaCl = salt addition; Cl₂ = chlorine addition; Alum = alum addition; Cl₂/Alum = combination of chlorine and alum addition
lysis and activate cellular enzymes, and a treatment period of 5 h. This second group was included to quantify the change in alkene concentration following NaCl addition and short-term storage.

The high dissolved organic carbon (DOC) of 28 mg·L\(^{-1}\) associated with the algal culture rapidly consumed the 1.8 mg·L\(^{-1}\) of chlorine that was applied. Therefore, the same experimental protocol was applied to a similar algal assemblage, with the exception that a higher chlorine dose of 24.5 mg·L\(^{-1}\) was used. This dose was selected to provide a chlorine residual for approximately 1 h. Concurrent analyses confirmed that 6.0, 0.8 and 0.02 mg·L\(^{-1}\) chlorine were present after 10, 30 and 60 min, respectively.

**Results and discussion**

**Effect of storage conditions on sample stability**

The results of the stability study for samples stored without headspace in darkened conditions at 4 °C are presented in Tables 3 and 4. The average values of four sample analyses are presented. Aldehydes in the unsalted samples were stable for short periods of up to 5 h. The addition of salt to the algal culture material releases and activates cellular enzymes, resulting in significant losses within 5 h. The increase in the *Asterionella*-derived octatriene concentration with both time and salt addition may result from slower liberation of PUFA through the frustule, combined with increased stability to biological oxidation.

When stored in abiotic reagent water, losses of heptadienal and decadienal were <20% for up to 336 h, whereas 78% of the added decatrienal was lost over the same period. When salt is added to the algal culture, virtually all the aldehydes are lost within 48 h. Even though these solutions were prepared with synthetic aldehydes, they are chemically equivalent and susceptible to enzymatic degradation.

Low biomass and organic content are typical of the Glenmore Reservoir. The long-term (10 y) average phytoplanktonic biomass is 0.24 mg·L\(^{-1}\) and DOC seldom exceeds 2 mg·L\(^{-1}\) (Satchwill, 2001). For these reasons, the storage conditions in reagent water are more representative of ambient conditions in the reservoir than the algal cultures. The stabilities of heptadienal and, to a lesser extent, decadienal under these conditions is in sharp contrast to that of decatrienal. This provides a plausible explanation as to why finished water T/O events are associated with blooms of *Uroglena* but not with blooms of *Dinobryon*. The heptadienal that results from *Uroglena* is stable through the water treatment process and into the distribution system. In the case of *Dinobryon* blooms, the finished drinking water is not always affected, even though raw water concentrations of decatrienal are well above the OTC.

Geosmin and 2-MIB were also present in the solutions and were measured concurrently. No losses of these compounds were observed over the 336 h storage period in either the biological matrix or reagent water. The addition of salt to biological matrix reduced geosmin and 2-MIB by 18 and 29%, respectively, following 48 h storage. As with the alkenes, the lower recoveries are likely due to the release of cellular enzymes.

**Table 3** Stability of alkene AVOC in stored algal cultures (n = 4)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Salt</th>
<th>Media</th>
<th>Heptadienal % change in concentration (relative standard deviation)</th>
<th>Decadienal</th>
<th>Decatrienal</th>
<th>Octatriene</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>–</td>
<td>Algal culture</td>
<td>+12 (24)</td>
<td>–9 (15)</td>
<td>+6 (4)</td>
<td>+230 (38)</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>Algal culture</td>
<td>–64 (31)</td>
<td>–81 (35)</td>
<td>–73 (20)</td>
<td>+505 (2)</td>
</tr>
</tbody>
</table>

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Effect of in-plant processes on mixed algal cultures

The results are summarised in Figure 1. The error bars represent ±1 standard deviation from the mean of three replicate analyses. As the measurements are of biological samples, the standard deviation pools both analytical error and true sample variability that arises from inhomogeneity at a cellular level and competitive biochemical processes.

The results of these experiments indicated that short-term storage of 5 h resulted in no significant change to the concentration of the aldehydes, and that the in-plant storage time will not remove or promote the formation of these T/O compounds. The addition of NaCl during the storage phase of the experiment resulted in lower recoveries for all three aldehydes. The recoveries of heptadienal, decadienal and decatrienal were reduced to 36, 9 and 27%, respectively, when compared to the control samples. The concentration of decadienal present in the samples was much lower than for the other compounds, possibly accounting for the higher relative loss following treatment.

Chlorine addition at 1.8 mg·L\(^{-1}\) resulted in decreased recoveries of heptadienal, decadienal and decatrienal to 66, 53 and 79%, respectively, when compared to the control samples. The degree of attenuation was commensurate with the small amount of chlorine added relative to the high DOC content of 28 mg·L\(^{-1}\) in the algal mixture. No measurable free chlorine was present 2 min after addition. At the higher chlorine rate of 24.5 mg·L\(^{-1}\), and with a chlorine residual present for the first hour of storage, the levels of all aldehydes were virtually eliminated and reduced by 98–100%. Although these conditions are

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Salt</th>
<th>Media</th>
<th>Heptadienal % change in concentration (relative standard deviation)</th>
<th>Decadienal</th>
<th>Decatrienal</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td></td>
<td>Algal culture</td>
<td>–100 (0)</td>
<td>–94 (123)</td>
<td>–100 (0)</td>
</tr>
<tr>
<td>336</td>
<td></td>
<td>Algal culture</td>
<td>–92 (134)</td>
<td>–96 (119)</td>
<td>–93 (115)</td>
</tr>
<tr>
<td>336</td>
<td></td>
<td>Reagent water</td>
<td>+23 (10)</td>
<td>–15 (5)</td>
<td>–78 (4)</td>
</tr>
</tbody>
</table>

**Table 4** Stability of synthetic T/O compounds in culture material and reagent water (n = 4)

**Figure 1** Effect of treatment processes on AVOC concentration in mixed algal cultures. Control samples with no treatment (NT). Treatments are: 5 h storage (5 h); salt addition (NaCl); chlorine addition at 1.8 mg·L\(^{-1}\) (top row) and 24 mg·L\(^{-1}\) (lower row); alum addition at 16 mg·L\(^{-1}\) (Alum); chlorine and alum addition (Cl\(_2\)/Alum). Average of three samples plotted. Error bars indicate ±1 standard deviation.
artificial in that culture materials with high organic content were challenged with high chlorine dose, it does demonstrate that chlorine oxidation will affect these algal derived T/O compounds.

The addition of alum had the unexpected effect of enhancing recovery of heptadienal and decadienal by a 5- and 8-fold amount, while the measured concentrations of decatrienal were not significantly affected. Visual inspection of the samples indicated that colloidal and particulate materials were removed from the water column through coagulation. It is not clear if this process enhanced the apparent recovery through a decrease in matrix effects and competitive absorption, or if catabolic processes were diminished, and thereby improved the short-term stability of T/O compounds. Microscopic examination of the algae in ambient waters subject to similar coagulant treatments demonstrated that the level of coagulant applied has no deleterious effect on the integrity of the algae.

The observed effects from the addition of chlorine combined with alum were consistent with the individual treatments. The enhanced recoveries of T/O compounds by coagulant addition were offset by the attenuation from chlorine oxidation. At the lower chlorine dose of 1.8 mg·L\(^{-1}\), recoveries of heptadienal, decadienal and decatrienal were 210, 110 and 56%, respectively, when compared to the control samples. At the higher chlorine dose of 24.5 mg·L\(^{-1}\), the recoveries of heptadienal and decadienal were 5 and 13%, respectively, when compared to the control samples. Notably, the amount of decatrienal present after treatment was 56%.

Conclusions
We found significant differences in the response of different AVOCs to treatment, related to molecular weight and degree of unsaturation. Heptadienal is more stable to treatment plant process and storage conditions than decadienal, and decatrienal was found to be relatively ephemeral. These differences provide new insight into the persistence of fishy odours in finished water supplies following outbreaks of C7 alkene-producing algae (Uroglena) and absence of such odours from C10 producers (Dinobryon), which were consistent with historical Glenmore plant records of algal outbreaks, customer complaints and flavour profile analyses.

References


