ASU SCHOOL OF Life Sciences
ARIZONA STATE UNIVERSITY

Feasibility Study for Early Warning Systems for Algae-induced Tastes and Odors

Final Report

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Executive summary

Taste and odor issues in drinking water can significantly impact customer satisfaction and reduce customer confidence in the quality of the water supply. Many of the observed taste and odor problems identified in drinking water in central Arizona are attributed to cyanobacteria (blue-green algae). Several species of filamentous cyanobacteria (those species whose cells combine to form long chains or filaments) are known to release the two most common compounds associated with taste and odor events, 2-methylisoborneol (MIB) and geosmin.

This study investigated the link between cyanobacteria abundance and the levels of MIB and geosmin present in the water. Three primary, and eight secondary, locations in the Salt River watershed and in the Phoenix metropolitan canal system were sampled during the study. Cyanobacteria filaments were quantified using two methods: 1) analysis of cells filtered on slides using epifluorescence microscopy and 2) automated particle analysis of water samples.

Results suggested that there is a correlation between cyanobacteria abundance and MIB concentration. There was less evidence to indicate a similar relationship with geosmin production. A decline in the cyanobacteria population after a period of bloom activity was followed by a pulse in elevated MIB concentration approximately one month later. This relationship was most evident in Saguaro Lake, the last impoundment on the Salt River before water enters the water treatment facilities in the Phoenix metropolitan area.

We conclude from this feasibility study that it may be possible to provide early warning of taste and odor events produced by the compound MIB by monitoring the population abundance of filamentous cyanobacteria. This is particularly true for storage reservoirs where the strongest patterns were observed.

Introduction

Taste and odor issues in drinking water can significantly impact customer satisfaction and reduce customer confidence in the quality of the water supply. Many of the observed taste and odor problems identified in drinking water are attributed to cyanobacteria (blue-green algae). The compounds 2-methylisoborneol (MIB) and geosmin, both known to be released by cyanobacteria, affect the taste and odor of drinking water. Some cyanobacteria species are also responsible for producing the toxic alkaloid cylindrospermopsin, which is linked to organ damage in mammals when ingested in high quantities (Falconer and Humpage 2006). Additionally, this toxin has been identified as a possible carcinogen (Falconer and Humpage 2001). In recent years, the presence of cyanobacterial toxins in water supplies has been associated with livestock deaths and even human illness and death (Falconer 2005).

The potential health hazard presented by these toxins is a relevant issue for U.S. agencies and utilities as these cyanobacteria can readily be found in many lakes, reservoirs and rivers across North America. Many of these water bodies provide source water for drinking water supplies. Previous studies have found these species to be present in the reservoirs of central Arizona, which are a major water supply system for the Phoenix metropolitan area (Sommerfeld et al. 2003, Westerhoff et al. 2005). These reservoirs are fed by a combination of the Salt River watershed, the Verde River watershed, and Central Arizona Project water transported by a ~200 kilometer canal system from the Colorado River. In addition to water storage and hydropower, the storage reservoirs also have a great recreational value, including boating and fishing. Filamentous cyanobacteria such as *sp. Cylindrospermopsis*, *sp. Phormidium* and *Anabaena sp.*, which have been linked with MIB and geosmin production, have all been observed in these rivers and reservoirs.

While correlations have been identified with MIB levels and seasonal environmental conditions (Westerhoff et al. 2005), it is less clear if a specific relationship exists between the timing of cyanobacteria blooms and high MIB or geosmin levels. A more thorough understanding of the relationship between algal population dynamics and increased levels of MIB and geosmin could provide early warning of taste and odor events. This advanced notice would benefit those utilities responsible for water quality by allowing them to respond to the imminent arrival of these nuisance compounds at their water treatment facilities.

This study was commissioned to further investigate the link between these taste and odor producing organisms and the compounds they produce. In undertaking this study we monitored Saguaro Lake, a storage reservoir, the Salt River and several canals in the Phoenix metropolitan area for the presence of filamentous cyanobacteria over a two month period. The assessment of the samples collected included the examination of water samples to determine the density of filamentous cyanobacteria as well as the chemical analyses of samples to measure MIB and geosmin concentrations. Cyanobacteria filaments were quantified using two methods: 1) epifluorescence microscopy of cells filtered onto membrane filters and 2) automated particle counting of water samples using a FlowCAM® particle analyzer (Fluid Imaging Technologies). This side-by-side comparison was intended to assess how the availability of automated technologies may contribute to simplifying and speeding up the process of monitoring water supplies by agencies and utilities.

Materials and methods

Primary sampling sites

A number of studies (both previous and ongoing) have been conducted on the Salt River watershed to examine the ecology of the algal populations and the presence of taste and odor compounds in the water supply. Several sampling sites used in previous studies were identified as suitable candidates for this study. Three sites were chosen as primary sampling sites: Saguaro Lake, the Salt River north of Blue Point Bridge on the Bush Highway, and the South Canal west of the Granite Reef Diversion Dam (Figure 1). Samples were collected from these sites on fifteen sampling visits conducted during the period of the study between 8/20/2009 and 10/16/2009.

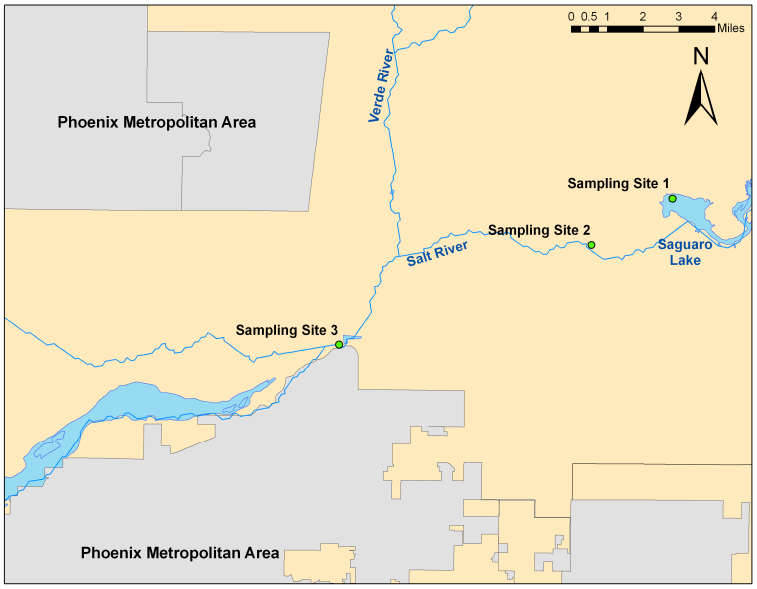

Figure 1 - Map of central Arizona showing the three primary sampling sites on the Salt River that were used for this study.

Site 1 – Saguaro Lake. This reservoir (maximum surface elevation 467m) is the last major impoundment on the Salt River before water supplies reach water treatment facilities in the Phoenix metropolitan area (Figure 2). The reservoir is officially 16 km long and has a maximum capacity of 86.1 million m^3, but this includes the upriver stretch of the Salt River that feeds into the lake. The reservoir basin itself is approximately 2.0 km by 1.0 km and has a hydraulic height (normal operating depth) of 35 m.

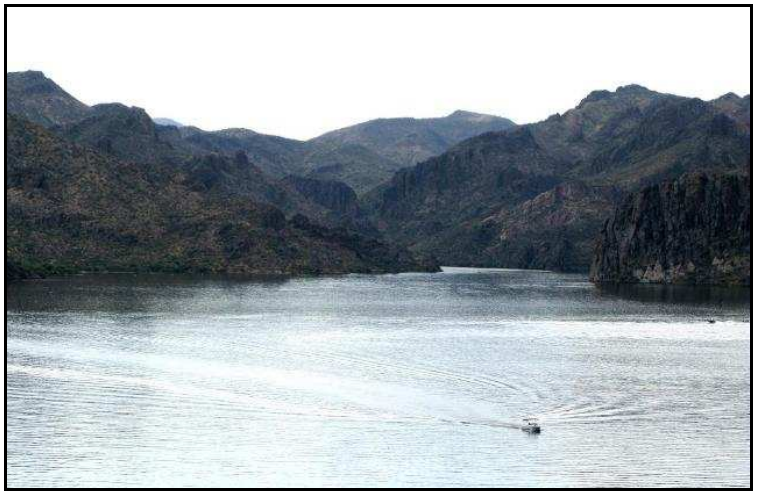

Figure 2 - Sampling Site 1, Saguaro Lake reservoir (central Arizona) on the Salt River (image: Harry Nelson, Fluid Imaging Technologies)

Site 2 – Salt River at Blue Point Bridge. This location is on the stretch of the Salt River that connects Saguaro Lake to the canal system that supplies the water treatment plants (Figure 3). The river is approximately 40 meters wide at this point, but as discharge is controlled at Stewart Mountain Dam by SRP, the river flow is somewhat detached from environmental conditions. During the period of the study, river flow ranged from 9.2-47.4 m^3/s, with a mean flow of 29.5 m^3/s.

Figure 3 - Sampling Site 2, the Salt River (central Arizona) 100 meters downstream of Blue Point Bridge (image: Harry Nelson, Fluid Imaging Technologies).

Site 3 – South Canal. This site is at the head of the canal just below Granite Reef Dam (Figure 4). This canal is fed by a combination of Salt River, Verde River and CAP canal water depending on the time of year and consumer demands. From this canal, water is diverted to secondary canals and then to lateral channels through which water is delivered to farms and cities in central Arizona. The canal is approximately 18 meters wide, and during the study, flow rates ranged from 16.8-25.7 m^3/s, with a mean of 21.1 m^3/s. For the majority of the study

period, the primary water source for the South Canal was the Salt River. However, this balance changed around the end of September when SRP increased the proportion of Verde River water entering the canal.

Figure 4 – Sampling Site 3, SRP South Canal (central Arizona) downstream of Granite Reef diversion dam (image: Philip Tarrant).

Secondary sampling sites

In addition to the water samples obtained from the primary sampling sites, samples were obtained from periodic sampling conducted by the Westerhoff laboratory at Arizona State University. Dr. Westerhoff's group samples the lakes, canals and rivers in central Arizona as part of an ongoing study to monitor taste and odor events in the Phoenix metropolitan water supply. Samples were provided on three occasions during the study from eight additional locations. These locations included the inflow and outflow of three metropolitan water treatment plants, the Arizona Canal and the Salt River.

Field sampling

Samples were collected from all three sample sites on each occasion. Surface water temperature was measured at each site using a YSI model 85 probe. Clean sampling bottles were rinsed twice with source water before being filled. They were then stored in a cooler for transport to the ASU laboratory. In the laboratory, samples were divided for testing using epifluorescence microscopy and the FlowCAM particle analyzer. All samples were processed within 24 hours of collection. Samples for MIB and Geosmin analysis were stored with zero headspace in 100ml vials and refrigerated until analysis by the Westerhoff lab.

Epifluorescence microscopy

Samples were processed as per Neuer and Cowles (1994). Each sample was fixed with 0.1 ml gluteraldehyde and then stained with 0.2 ml DAPI. A quantity of 1-15 ml of sample was filtered onto black polycarbonate filters. These filters were then placed on a labeled slide, embedded in immersion oil and covered with a cover slip. Slides were then viewed under a blue light and UV excitation and organisms were logged by taxonomic group. Digital images of each sample were recorded for archival purposes

FlowCAM analysis

The FlowCAM particle analyzer can operate in three different image acquisition modes – 2 Channel Fluorescent Triggering (triggered by the presence by Chlorophyll and/or Phycoerythrin), Forward Scatter Triggering, or Auto Imaging. The different modes are available to handle the different characteristics of the sample(s) to be analyzed. For this study all samples were processed using the Auto Imaging mode in conjunction with a 100 μm flow cell and a 10X objective lens. For the samples taken on the Salt River (Sites 1, 2, and 3), 2ml of sample was processed on each occasion. The samples supplied by the Westerhoff group from the Phoenix metropolitan area canal system generally exhibited lower organism densities. Consequently, a sample quantity of 10ml was processed to ensure that sufficient numbers of particles were observed by the analyzer.

MIB and geosmin analysis

MIB and geosmin concentrations were determined with the support and assistance of Dr. Westerhoff's group. This group conducts ongoing research into the effect of several compounds, including MIB and geosmin, on the quality and taste of drinking water supplies and is equipped for assessing MIB and geosmin concentrations.

Compounds were separated from a 25ml water sample using solid phase microextraction (SPME). The sample obtained was heated to 60-70°C and stirred. The solubility of the compounds was decreased by adding 25% w/v sodium chloride. The compounds were then adsorbed by a tri-layered coating of Divinylbenzene/Carboxen/polydimethylsiloxane fiber. The extracted compounds were desorbed from the fiber in the glass injection liner of a gas chromatograph-mass spectrometer at 250°C. Compound identification was then achieved by comparing retention times and primary ion masses to previously measured standards. Analyte concentration was calculated from area responses relative to external calibration standards.

Results

Sample processing times

One goal of this study was to compare the differences between microscopic sample analysis and automated particle counting. Table 1 shows estimates of sample processing times. These estimates are only intended as a guide to processing times and may vary dependent on sample volumes and equipment used. Sample processing times are indicated along with an indication of task type, defined as either a one-off activity or an ongoing task.

Table 1 - Estimates of time required to analyze water samples in order to produce approximate cell counts of algal organisms (using traditional microscopy counts and FlowCAM particle analysis) and estimates of MIB and geosmin concentrations.

Task	Approximate Time	Continuing/Initial Activity
Epifluorescence Microscopy		
Sample Filtering (per sample)	20 minutes	Continuing
Slide Preparation (per sample)	10 minutes	Continuing
Slide Analysis (per sample)	30-45 minutes	Continuing
FlowCAM Method		
Filter Library Creation	Basic filter 20hrs, Complex filter 40hrs	Initial
Sample Processing (per sample, varying by sample volume)	10-30 minutes	Continuing
MIB/Geosmin Concentration		
Solid Phase Microextraction (per sample)	30 minutes	Continuing
Gas Chromatograph Processing (per sample)	25 minutes	Continuing

Water sources and flow rates

In general, the source water for the three primary sampling sites was the Salt River. This was exclusively the case for the Saguaro Lake and Salt River sites. However, the water samples collected from the South Canal were influenced by water released from Bartlett Dam on the Verde River, which has its confluence with the Salt River downstream from Blue Point Bridge, but upstream from the canal. For the first half of the study the main water source for all three sample locations was the Salt River system. During this period it is estimated that less than 10% of the water at the South Canal originated from the Verde River. However, towards the end of September 2009, SRP began its normal change over from Salt River water to Verde River water. Around 9/20/2009, the flow rates from Stewart Mountain Dam began to decline and Verde River water increased as a proportion of the total entering the canal system (Figure 5). On 9/30/2009, Verde River water contributed more than 50% of the total water entering the South Canal and remained at a level between 58% and 74% for the remainder of the study.

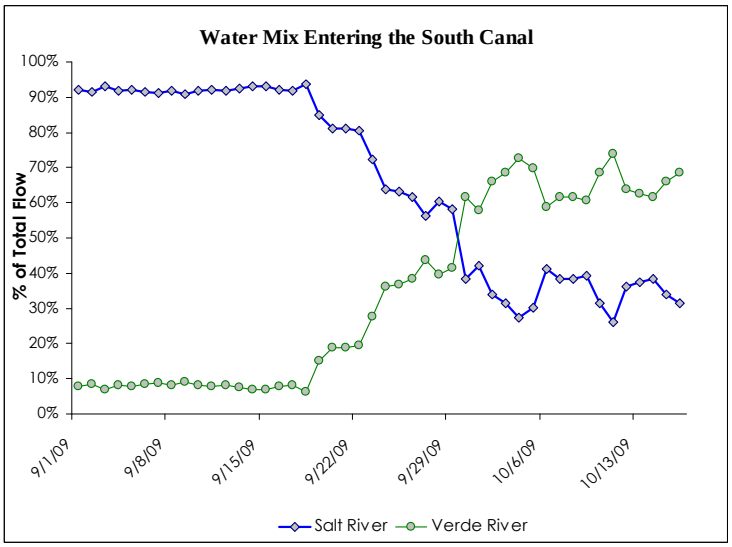

Figure 5 – Proportion of source water entering the South Canal via the Granite Reef Diversion Dam for the period September 1 to October 16, 2009. As the Salt River water volume declined, it was replaced by Verde River water, which dominated the water volume entering the South Canal around September 30 2009 (source: Salt River Project, www.srpnet.com).

Water released from Stewart Mountain Dam at Saguaro Lake influenced the flow rate at the Salt River and South Canal sampling locations. SRP manages the water volume diverted into the South Canal based on consumer demand by means of the diversion dam complex at Granite Reef. This diversion dam is just upstream of the sampling location used in this study. During this study the flow rate in the South Canal ranged from a minimum of 16.8 m^3/s to a maximum of 25.7 m^3/s. By comparison, the flow rate in the Salt River varied between 9.2 m^3/s and 47.4 m^3/s, (Figure 6).

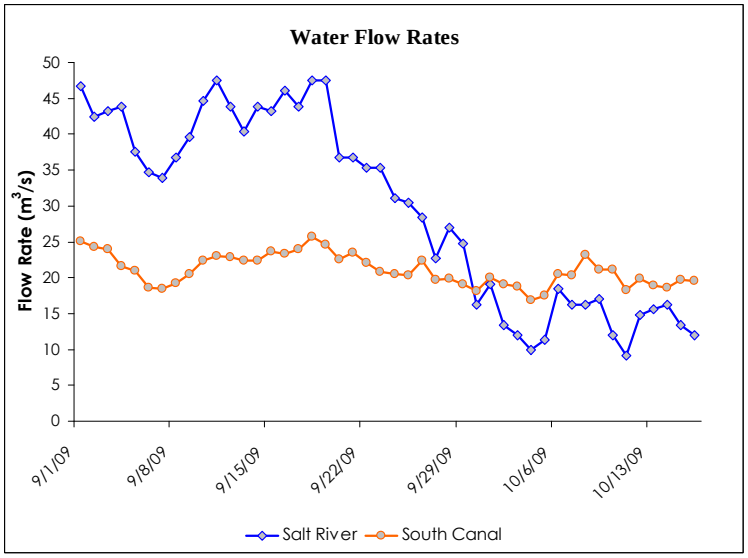

Figure 6 – Water flow rates for the Salt River and South Canal for the period September 1 to October 16 2009. Water released from Stewart Mountain Dam declined substantially over this period, although the volume diverted into the South Canal was less variable. (Source: Salt River Project, www.srpnet.com).

Water temperature

The observed water temperature at each sample location was in the range expected for the time of year. The temperature measured at the surface in Saguaro Lake at the beginning of the study was 29.4°C. Temperature declined during the study period, reaching a minimum of 21.8°C by the termination of sampling activities in mid-October. Temperatures in the Salt River and South Canal were typically several degrees cooler (Figure 7).

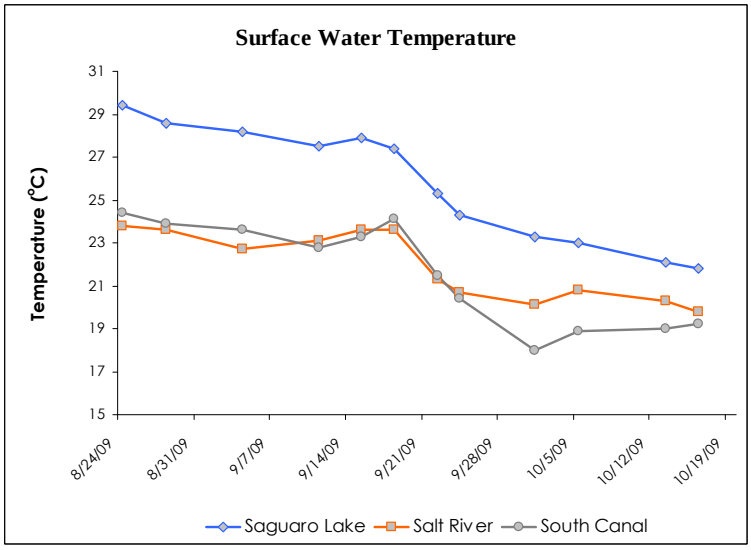

Figure 7 – Surface water temperature for three locations (Saguaro Lake, Salt River, and South Canal) for the period August 24 to October 16, 2009.

This difference in water temperature is to be expected as the Stewart Mountain Dam outlet is near the base of the dam. As a result, water released from the reservoir at this time of year is normally somewhat colder than the reservoir surface water due to the presence of a thermally stratified water layer that develops over the summer months. Seasonal changes in weather normally contribute to an overturning of Saguaro Lake between mid-September and early October, causing a reduction in the degree of thermal stratification in the upper 15-20m of the lake. This appeared to occur towards the end of September with the surface water temperature decreasing ~5°C during the second half of the month.

Algal population abundance

During the study period, the Salt River system showed signs of a bloom of several filamentous cyanobacteria. The timing of this bloom was fortuitous because it ensured that a good density of algal organisms was available for analysis by both quantification methods used in this study. The high numbers of cyanobacteria present in the river system over the course of the study also resulted in the elevated levels of MIB and geosmin observed during our study periods (see below).

Particle concentration determined with the FlowCAM in samples taken from the Salt River sites varied from a minimum of 3439 particles/ml (observed 10/5/2009) in the Salt River at Blue Point Bridge to a maximum count of 210893 particles/ml observed in Saguaro Lake (9/11/2009)(Figure 8). Particle concentration was consistently higher in Saguaro Lake than at either of the other two primary sampling sites. In addition to higher concentrations of

organisms overall, the Saguaro Lake site also had the highest proportion of filamentous cyanobacteria, ranging from 20% (10/9/2009) to 48% (9/4/2009) with a mean of 34% of the sample population. By contrast, filamentous cyanobacteria in the Salt River and South Canal only constituted a mean of 13% and 3%, respectively, of the total particle count.

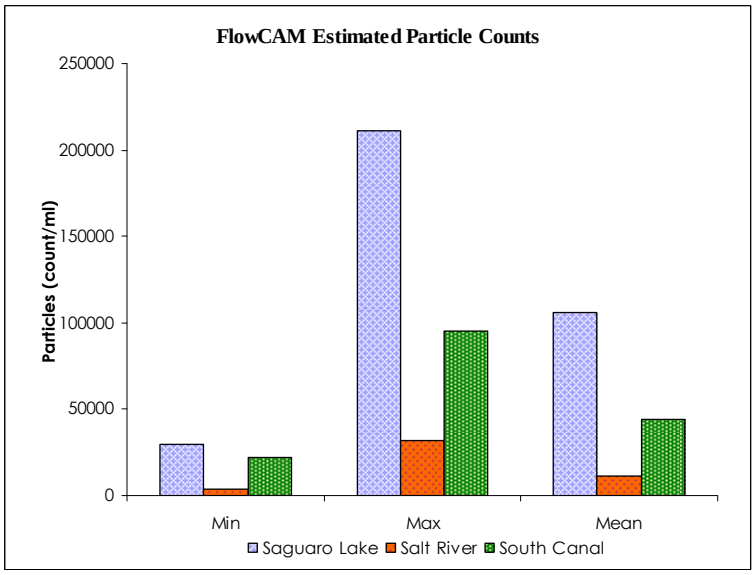

Figure 8 – Minimum, maximum and mean particle counts (per milliliter) as estimated by the FlowCAM particle analyzer for three locations (Saguaro Lake, Salt River, and South Canal) in central Arizona.

Samples collected by the Westerhoff group from the canal system generally showed lower concentrations of both total particles and filamentous cyanobacteria. The minimum particle count was 893 particles/ml with a maximum of 38059 particles/ml and a mean of 12249 particles/ml. The mean proportion of filamentous cyanobacteria was approximately 5%.

Composition of filamentous cyanobacteria

In order to prepare a time-series of algal species present during the whole study period, we counted the full sequence of microscope slides prepared from Saguaro Lake samples. Our analysis of these slides using epifluorescence microscopy focused on the filamentous cyanobacteria that dominated the algal population. Four genera/species of filamentous cyanobacteria could be distinguished in measurable quantities during the early stages of the algal bloom in August through to early September. These primary bloom formers were *Cylindrospermopsis raciborskii*, *Cylindrospermopsis curvispora*, *Aphanizomenon sp.* and *Phormidium sp.* (Figure 9). *Phormidium sp.* and *Cylindrospermopsis raciborskii* made up 28% of the total cyanobacteria (14 % and 14%, respectively). The counts of filamentous cyanobacteria produced by microscopic examination and those produced by the FlowCAM were in good agreement (Figure 10). While some variation existed between sample estimates, this difference was not significant (t-test, DF=23, p=0.89).

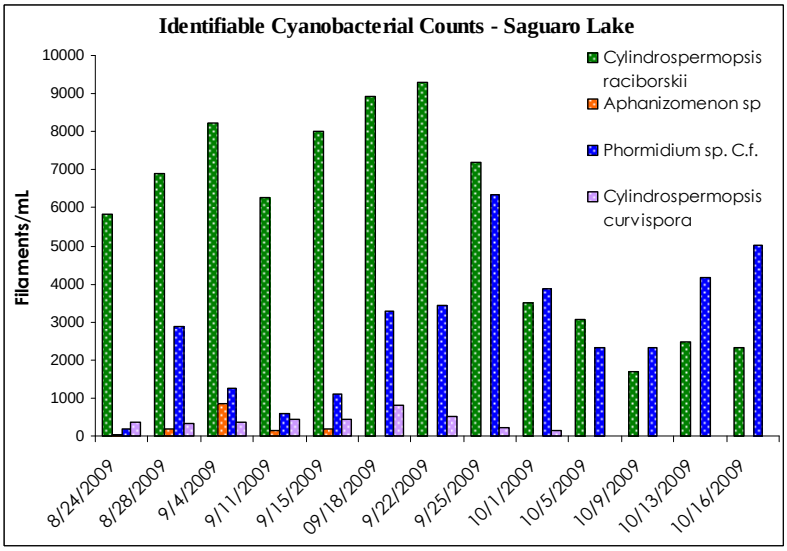

Figure 9 – Filament counts of four identified cyanobacteria genera/species (filaments/ml), *Cylindrospermopsis raciborskii, sp. Aphanizomenon, sp. Phormidium, and Cylindrospermopsis curvispora*, observed in Saguaro Lake, AZ.

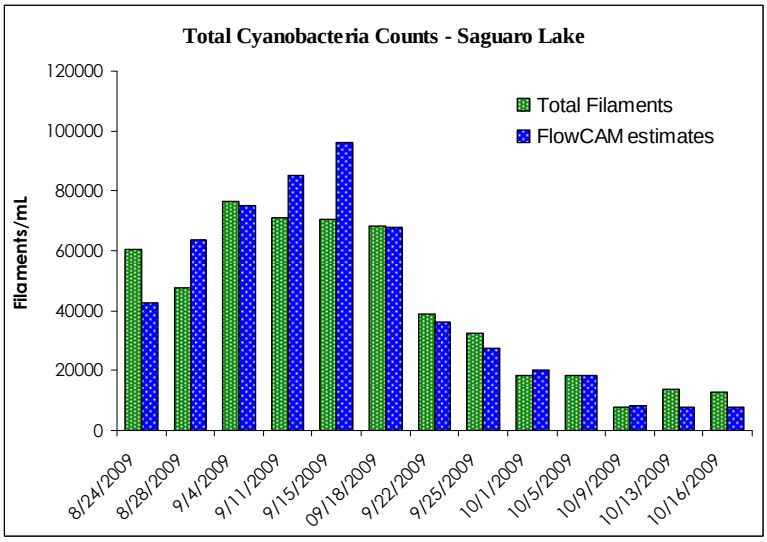

Figure 10 – Numbers of total cyanobacteria (filaments/ml) observed during the study period using epifluorescencemicroscopy for Saguaro Lake, AZ. Concomitant FlowCAM particle analysis estimates are included for comparison.

MIB and geosmin levels

During the study period, MIB levels varied substantially, and the three primary test sites showed significant differences in MIB concentrations (Figure 11, Figure 12 and Figure 13). MIB concentrations ranged from a minimum of 8.3 ng/L observed in the South Canal to a maximum of 138.0 ng/L in Saguaro Lake. Saguaro Lake generally had higher MIB concentrations than either of the other two locations with a mean concentration of 47.2 ng/L, compared to 20.2 ng/L and 16.6 ng/L for the Salt River and South Canal, respectively. By contrast, geosmin levels were generally much lower at all three sample sites, with values

ranging from 2.3 ng/L to 9.2 ng/L and mean values of 6.9 ng/L in Saguaro Lake, 3.2 ng/L for the Salt River and 3.8 ng/L in the South Canal.

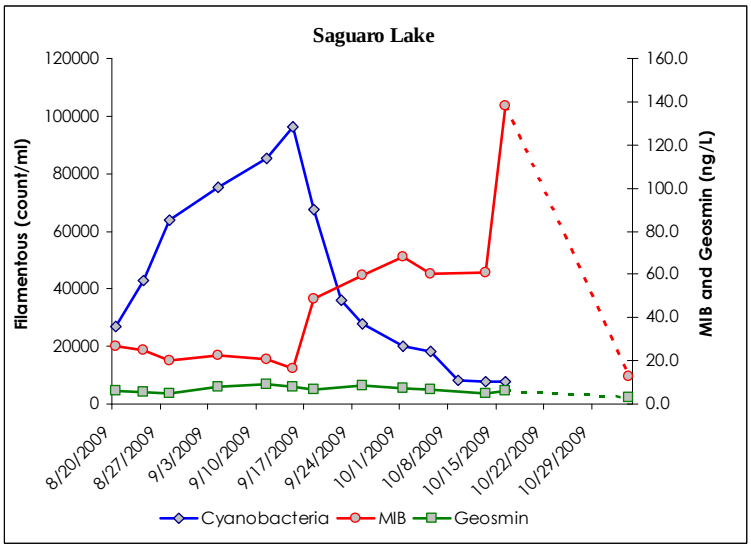

Figure 11 – FlowCAM estimates of filamentous cyanobacteria (count/ml) compared to levels of MIB (ng/L) and geosmin (ng/L) for Saguaro Lake in central Arizona for the period August 20 to October 16 2009. Dotted lines indicate the decline in MIB/geosmin levels after study period was over as measured by regular monthly sampling (on 11/2/2009) conducted as part of a separate study.

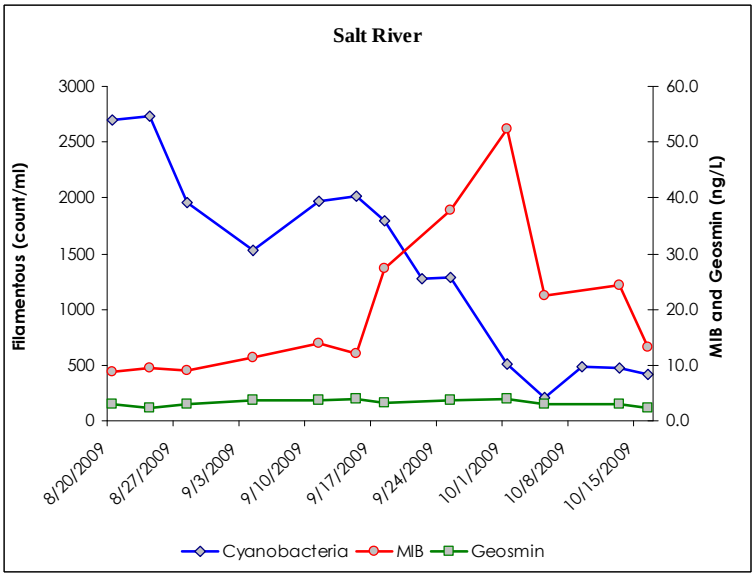

Figure 12 – FlowCAM estimates of filamentous cyanobacteria (count/ml) compared to levels of MIB (ng/L) and geosmin (ng/L) for the Salt River for the period August 20 to October 16 2009.

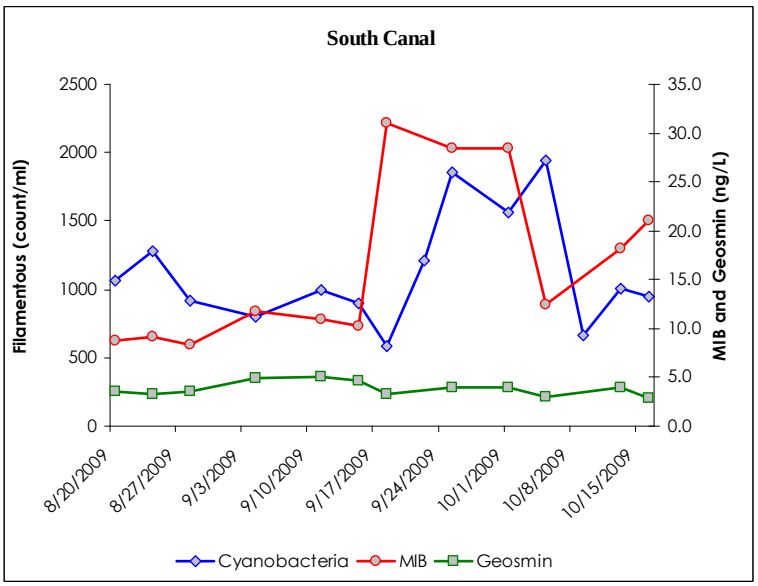

Figure 13 – FlowCAM estimates of filamentous cyanobacteria (count/ml) compared to levels of MIB (ng/L) and geosmin (ng/L) for the South Canal for the period August 20 to October 16 2009.

In Saguaro Lake and the Salt River, increases in MIB coincided with the decline in cyanobacteria, and concomitantly with the decline in water temperature. This pattern was not repeated in the South Canal, where there was a period of increased MIB concentration that coincided with an increase in the cyanobacteria filament count. However, the observed increase in both MIB and cyanobacteria counts occurred concurrently with the change from Salt River to Verde River water.

In Saguaro Lake and the Salt River, no measurable real-time correlation between filamentous particle counts and MIB level (r^2=0.024, n=35) could be observed. In fact, the peak in MIB concentrations occurred some time after the highest observed concentration of filamentous cyanobacteria (Table 2). This delay was approximately one month for Saguaro Lake and six weeks for the Salt River. Again, this pattern was not reflected at the South Canal site. It is noted, however, that geosmin concentrations did show evidence of correlation with concomitant particle counts (r^2= 0.63, n=54).

Table 2 – Dates of highest measured filamentous particle counts (count/ml) compared to highest measured concentrations of MIB for three sample sites on the Salt River in central Arizona.

	Saguaro Lake	Salt River	South Canal
Date of highest filamentous particle count	9/15/2009	8/24/2009	10/05/2009
• Filamentous particles/ml	96393	2739	1939
• MIB	16.3	9.5	9.2
• Geosmin	8.1	2.3	3.3
Date of highest MIB value	10/16/2009	10/01/2009	9/18/2009
• Filamentous particles/ml	7805	6586	588
• MIB	138.0	52.4	31.1
• Geosmin	6.3	4.0	3.3

Regression analysis of limited data available from the only static water body (Saguaro Lake) suggested that during the early stages of the cyanbacteria bloom, when nutrient conditions and temperature were presumably ideal, there was an inverse correlation between the increase in cyanobacteria and MIB levels (r^2=0.66, n=6). An inverse relationship was also evident as the cyanobacteria population declined and MIB concentration increased (r^2=0.82, n=5).

Geosmin levels during this study were much lower and less variable than MIB concentrations. Maximum measured geosmin levels were only 188% of minimum compared to a range of 847% for MIB. Both of these minimum-maximum ranges occurred in Saguaro Lake.

Discussion

MIB production in cyanobacteria

Previous studies have linked a number of filamentous cyanobacteria species, including *Cylindrospermopsis, Phormidium, Pseudanabaena, Anabaena* and *Oscillatoria*, with the production of the taste and odor producing compounds MIB and geosmin (Izaguirre and Taylor 1998, Rosen et al. 1992, Sommerfeld et al. 2002, Sugiura and Nakano 2000, Zimmerman et al. 1995). The timing of MIB and geosmin production by these various species has been linked to air and water temperature (Tung et al. 2008, Westerhoff et al. 2005). It is also recognized that other influences, such as nutrient constraints, nutrient influx events, light intensity and competition, may result in the release of MIB by these organisms (Saadoun et al. 2001). Although these environmental factors appear to affect MIB and geosmin production by filamentous cyanobacteria, this study focused on the simplest potential relationship, that between population and MIB/geosmin concentrations.

MIB and geosmin levels in relation to cyanobacteria abundance

Our results indicate that there may be a definable relationship between cyanobacteria population levels and concentrations of these nuisance compounds. In two of the three primary sampling locations we noted that increases in MIB levels were observed after the cyanobacteria bloom began to decline. This suggests that the release of MIB may result from the stress and/or decomposition of these algal species, which has been noted in other studies (e.g., Juttner 1995).

In Saguaro Lake this increasing trend began within a few days of the apparent peak in cyanobactia abundance and steadily increased as the filamentous cyanobacteria count continued to subside. The highest observed MIB concentration (138.0 ng/L) was recorded on the last day of sampling on 10/16/2009. Although the MIB concentration may have increased further, by the time the regular monthly sample was taken on 11/2/2009 by Dr. Westerhoff's group, the MIB levels had dropped significantly (~13 ng/L). This pattern was repeated in the Salt River, where the population appears to have peaked around the beginning of our study. MIB concentrations rose gradually into September and then increased steeply as the decrease in particle count continued. We believe that a drop in water temperature may have contributed to the acceleration in MIB concentration at this time. While there appears to be an increase in MIB concentration in the South Canal in response to the declining algal population (around 9/17/2009), this trend was masked by an increase in particle count that began around

9/22/2009. We believe this increased count originated in the Verde River water being diverted into the canal at this time.

Of the total cyanobacteria population identified in Saguaro Lake, 27.7% was comprised of two well known MIB producers, *Phormidium sp.* (14.0%) and *Cylindrospermopsis raciborskii* (13.7%) (Katsuhiro et al. 2004, Sommerfeld et al. 2002, Sugiura et al. 1997, Westerhoff et al. 2005). However, it is generally recognized that many species of filamentous cyanobacteria produce these compounds, so it is likely that other unidentified species in our samples also contributed to the MIB concentrations.

Monitoring programs for filamentous cyanobacteria populations

For organizations interested in managing taste and odor issues there are potential benefits to be realized by monitoring the source water supply on a regular basis. By tracking changes in the algal population density we have the opportunity to identify the peak bloom conditions that can act as a pre-cursor for taste and odor events. The necessary information can be obtained by either the use of traditional sampling, filtering and cell counts using microscopy, or by utilizing an automated technology such as the FlowCAM particle analyzer.

Both these methods require the collection of water samples and the ongoing cost of such a program would vary by geographic location and the resources and methods used for sample collection. For some water supply scenarios it would be appropriate to sample in the locality of the water treatment facility, whereas in others the supply reservoir would be a more appropriate location. In either case a period of pre-testing would probably be required to determine the dynamics of the individual system and to identify appropriate lead times for bloom conditions as an indicator of elevated MIB or geosmin levels.

There are different issues associated with monitoring water supplies dependent on which method is adopted. Agencies choosing to use microscopy may expect to invest more time in sample processing on a sample by sample basis. As a manual method, this technique requires "hands on" processing at all stages of the process. Additionally, taxonomic identification of species requires technicians to have received the necessary training or education.

Organizations choosing to adopt the automated technologies now available may be able to process samples with less experienced personnel. Also, the FlowCAM, and presumably other similar technologies, can be left unattended for short periods of time. This would allow personnel to conduct other tasks in parallel with sample processing. The use of this technology, however, requires an upfront investment in the development of "filters" capable of isolating target organisms. While these filters are reasonably effective in identifying filamentous particles they cannot easily isolate curved or spiral cyanobacteria filaments. Also, it is unlikely in our view that filters could operate at a species level, although this is not necessarily an issue as so many filamentous cyanobacteria species are known MIB producers. However, all images processed by the particle analyzer are retained and can be further examined by the operator if taxonomic classification is required.

In both cases, the cost of providing an ongoing monitoring program requires an investment in trained personnel and the necessary capital equipment, whether that is an epifluorescence microscope or a particle analyzer.

Potential for early warning of elevated MIB concentrations

The indication that MIB levels may be tracked by monitoring the population levels of resident filamentous cyanobacteria is encouraging. However, further study would be required to test this hypothesis. It is likely that even if population trends are indicative of pending MIB activity, the temporal relationship between bloom peaks and elevated MIB concentration would vary based on the dynamics of individual water supply systems. These individual characteristics would need to be accounted for in determining the timings of taste and odor events. Regular monitoring of high temporal resolution (every few days) similar to that conducted during this study would be required in order to accurately identify the highpoint of an algal bloom. Assuming the findings of this study are confirmed, we could expect that receiving early warning of elevated MIB concentration using similar methods is an achievable goal. Early notification of potential taste and odor events may be of particular interest to those parties interested in managing water quality.

Potential for early warning of elevated geosmin concentrations

The lack of correlation between geosmin concentrations and filamentous particle counts observed in this study suggests that there may not be a time lag between algal bloom conditions and geosmin production. The data indicate that geosmin may be released by healthy cells, but that geosmin production may increase proportionately as a cyanobacteria population declines. Unfortunately, this relationship would only be confirmed by a longer term study of the physiology of the cyanobacteria.

Conclusions

The release of MIB and geosmin by filamentous cyanobacteria can be triggered by several factors. The interaction between different organisms, nutrients and environmental conditions may all combine to influence the timing of MIB and geosmin production. This study did, however, observe a pattern in the release of MIB relative to the algal population density. If this pattern proves to be repeatable, then it is reasonable to expect that location specific monitoring programs may be able to provide early warning of taste and odor events based on the observation of algal bloom conditions. The good agreement observed between the traditional laboratory/microscopy method and the particle analyzer method suggest that additional choices now exist for agencies and utilities tasked with designing and implementing this type of monitoring program. The potential early warning provided by tracking the cyanobacteria population in this way should allow water treatment facilities to plan and prepare for the reductions of these compounds using the industry standard methods currently available. It is less clear if this outcome can be achieved for geosmin releases due to the less distinctive patterns observed in the data collected so far.

Guidance to Utilities: Monitoring Cyanobacteria Abundance for Early Warning of Taste and Odor Events

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Introduction

Taste and odor issues in drinking water can significantly impact customer satisfaction and reduce customer confidence in the quality of the water supply. Many of the observed taste and odor problems identified in drinking water are attributed to cyanobacteria (blue-green algae). The two most common compounds normally associated with taste and odor events are 2-methylisoborneol (MIB) and geosmin, are known to be released by cyanobacteria, particularly filamentous species. i.e. those species whose cells combine to form long chains or filaments.

A variety of cyanobacteria genera are present in the lakes, reservoirs and rivers of North America. Where these water bodies are used to provide water for drinking supplies, the release of these taste and odor compounds is an issue for those agencies responsible for the quality of drinking water. Taste and odor events can occur without warning, and once identified can require expensive management measures in order to reduce their impact on the perceived quality of the final product.

Monitoring the source water supply such as the storage reservoir may provide the opportunity to identify the precursors to a taste and odor event. While the early warning of the production of these compounds will not reduce the cost of corrective measures, it can increase the preparation time available to the water treatment plants and allow them to deploy their resources more effectively.

This document is intended to provide guidance to AWWA members who wish to conduct an ongoing monitoring program for the presence and abundance of filamentous cyanobacteria. This guidance is based on a field study conducted on the Salt River watershed in central Arizona, which identified a relationship between the timing of a cyanobacteria bloom and the elevated levels of MIB that were released as the algal population declined. The lack of a similar observed trend in geosmin production suggest that ongoing monitoring may be less likely to identify the imminent release of geosmin by these organisms.

Requirements of a monitoring program

Based on the principle that taste and odor compounds will be released after an algal bloom primarily comprised of cyanobacteria, it is important for a monitoring program to produce regular data that will allow investigators to identify the incipient bloom and then isolate the peak in cyanobacteria abundance. This peak in algal abundance, followed by the inevitable population decline, appears to be a precursor to MIB production. In addition to the provision of regular data, it is also necessary to have the basic taxonomic recognition skills available to ensure the correct identification of the species most likely to produce these compounds.

Finally, having the capability (if available) to chemically analyze samples for the presence of MIB and geosmin can provide confirmation of the taste and odor event when it occurs.

Issues for consideration

MIB production has been linked with a number of environmental factors that can trigger MIB releases. These include air and water temperature as well as other influences, such as nutrient availability and competition (Saadoun et al. 2001, Westerhoff et al. 2005). We believe that each geographic location will have different requirements for a monitoring program based on the relationships between the biotic and abiotic factors. Consequently, it is helpful to consider a number of questions about the ecology and environmental conditions, and to factor these into the program design, in order to ensure that the monitoring program delivers the desired outcome. This list is not intended to be exhaustive, but is intended to suggest areas that need to be examined as part of the preparation for monitoring the local water supply.

1. How far is the storage reservoir from the water treatment locations?
2. How is water released from the reservoir?
3. How is the water transported from the storage reservoir to the water treatment locations (river/canal/pipeline, etc)?
4. What are the flow rates in the transportation network?
5. What are the primary cyanobacteria species present in source water location?
6. When is the normal cyanobacteria bloom period in the source reservoir?
7. What is the temperature profile in the reservoir and supply system?

The answers to each of these questions will help to build a picture of the specific attributes of the local drinking water supply system. An understanding of these system attributes will help identify which factors have the most influence on the relationship between cyanobacteria abundance and elevated MIB levels.

Common taste and odor producers

Previous studies have identified a number of common culprits capable of producing MIB and geosmin (see below). If these cyanobacteria genera are present in a watershed, then they are likely to contribute to taste and odor events.

Genus	Geosmin	MIB	Morphology
Anabaena	X	X	Filamentous
Aphanizomenon		X	Filamentous
Cylindrospermopsis		X	Filamentous
Oscillatoria	X	X	Filamentous
Phormidium		X	Filamentous
Planktothrix	X	X	Filamentous
Pseudanabaena		X	Filamentous
Synechococcus	X	X	Unicellular

Sources: Badawy et al. 1999, Izaguirre and Taylor 1998, Sommerfeld et al. 2002, Sugiura et al. 1997, Sugiura and Nakano 2000.

Field sampling regime

To provide the temporal resolution necessary to determine when an algal boom is peaking, we recommend that field sampling should take place every 3-4 days. This frequency is sufficient to track the changes in algal concentration as well.

Analysis methods

Two analysis methods suitable for ongoing monitoring are: 1) conducting cell counts using epifluorescence microscopy of cells filtered onto membrane filters and 2) automated particle counting of water samples. In this study, the epifluorescence cell counts were conducted by a trained laboratory technician. The automated particles counts were produced using a FlowCAM® particle analyzer (Fluid Imaging Technologies). Both these methods produced statistically insignificant differences in the estimates of algal abundance.

Epifluorescence microscopy method

As per Neuer and Cowles (1994): samples should be fixed with 0.1 ml gluteraldehyde and then stained with 0.2 ml DAPI. The sample is then filtered (1-15 ml based on organism density) onto black polycarbonate filters. Once the filtration is complete, the filter is placed on a labeled slide, embedded in immersion oil, and covered with a cover slip. Prepared slides can be stored in a freezer until ready for viewing. Slides are viewed on a microscope under blue or UV light excitation. Organisms can be logged by taxonomic group and cell counts conducted to produce estimates of algal concentration. Additionally, using an appropriate camera, digital images can be recorded for archive purposes.

Semi-automated particle analysis

While it is not our intention to reproduce the user documentation supplied with equipment such as the FlowCAM, it is worth highlighting how the equipment was used in this study to monitor cyanobacteria abundance.

Equipment set-up (FlowCAM)

The FlowCAM can operate in three different imaging modes – 2 Channel Fluorescent Triggering (triggered by Chlorophyll and/or Phycoerythrin), Forward Scatter Triggering, or Auto Imaging. The different modes are available to adapt the FlowCAM to samples with different characteristics. i.e. samples of varying particle concentration.

For the samples processed during this study, the FlowCAM was set up as follows:

Objective lens – the 10x objective was the only one used due to the size of the filamentous cyanobacteria. At 20x magnification, many of the cyanobacteria will not fit entirely on the screen. This leads to parts of organism being lost through image cropping. The 4x objective was not suitable because it did not provide sufficient magnification to capture usable images of the cyanobacteria.

Auto image mode – we chose to use Auto Image Mode to capture particle images because we found that many cyanobacteria filaments we processed did not contain enough chlorophyll to trigger the laser in Trigger Mode. Auto Image Mode (in which a laser is not used) captures

every particle that possesses a contrasting edge sufficient to differentiate it from the background.

Sample volume selection – the sample volume processed was adjusted to the particle abundance. For high-particle abundances, the sampling volume was 2 ml, which allowed the collection of close to the maximum number of captured images that the internal FlowCAM software can process. At low particle concentrations, 10 ml of sample was processed, which provided a particle count high enough for statistical analysis.

Sample pre-filtering – All sample water was filtered through a 100µm Nitex filter. While this pre-filtration process enabled us to exclude larger debris particles, it did not prevent the passage of the majority of the cyanobacteria, as the filamentous nature of the species of interest allowed them to pass through the filter mesh.

Segmentation value – the segmentation value measures the difference in contrast between the background and a particle. Above the specified threshold, particles are captured as images, and are conversely omitted under this threshold. In order to capture images of the cyanobacteria, the Segmentation Threshold had to be lowered from "25" to "7", due to their low contrast with the background. Note: the Segmentation Threshold is composed of unitless values.

FlowCAM library and filter building parameters

To be able to focus sampling efforts on the filamentous cyanobacteria associated with MIB and geosmin production, it is necessary to develop a discriminating filter that can be applied when processing water samples. A filter is developed over time as part of ongoing processing activities, but it can take up to 20 hours of development to produce a filter with the necessary attributes to select the majority of filamentous cyanobacteria present in samples. Further time invested in filter development would increase filter effectiveness, but this time should be balanced by a "return on investment" consideration.

Library: Approximately four hundred images were selected from samples in Saguaro Lake, the Salt River, and the South Canal at Granite Reef. Consideration was given to selecting a "broad range" of images, including organisms across the spectrum of length, width, and color. A high variability existed amongst the filamentous cyanobacteria, which was included in the building of the image library.

Filter: When building the filter, it was common to find that many long, thin diatoms were confused with the filamentous cyanobacteria by the computer software. Using a combination of the parameters of "aspect ratio" (the ratio of height to length), length, width, and segmentation value (edge contrast), the filter was able to differentiate between most diatoms and cyanobacteria. All values were determined by the "average" for filamentous cyanobacteria. The measurements provided by the FlowCAM software are relative to other FlowCAM samples and cannot necessarily be compared with values obtained by other estimation methods.

Note: Additional parameters such as those of color would have been beneficial in the process of distinguishing diatoms from cyanobacteria. However, we were limited by the strong grayish

color gradient produced across the background, which affects overall color variances of each image. To date, a solution does not exist for this issue.

Additional notes on operation

1. Over time, the flow cell will degrade and become fouled. When this degradation occurs, imaging quality is affected and the flow cell needs to be replaced. We found that, on average, the flow cell needed to be changed once a month.

2. Regular cleaning of the flow cell, the objective lens, and the collimator lens will facilitate easier set-up and focusing of the camera. Additionally, the light transmittance is more consistent over time if the equipment is cleaned regularly.

3. Regular replacement of the peristaltic pump tubing will lead to more consistent flow rates through the flow cell. With regular replacement, we observed rates of approximately 0.49ml per minute through the equipment.

4. Between individual samples, the flow cell, tubing, and funnel were thoroughly rinsed with Milli-Q water. When cleaning the equipment this way, it is possible to pass a reasonable quantity of water through the flow cell using the Prime (Purge) "10" setting on the peristaltic pump.

5. Occasionally, it was noted that air bubbles or debris fouled the flow cell. When this occurred, drawing detergent through the system often remedied the situation. After cleaning with detergent, Milli-Q water can be passed through to remove any detergent residue.

FlowCAM image post-processing

The FlowCAM is equipped with VisualSpreadsheet™ software for instrument operation and post-processing analysis. Data is saved as an 'LST' file, which includes TIFF images of all particles imaged, with each image having up to 28 image parameters being collected. The software also has image recognition capability providing for the identification, classification, and enumeration of organisms of interest.

- Organize FlowCAM LST files by location and date. Ideally, a minimum sample size of 20 images of each species of interest is required to fully realize the FlowCAM's recognition capabilities.
- Create 'Image Libraries' of suspected taste and odor producing species from cultures and/or natural water samples.
- Utilizing the image recognition capabilities of the FlowCAM, identify and classify species of interest (taste and odor causing species) in the sample.
- Re-organize any mis-identified images into correct image libraries in order to improve ongoing image recognition capabilities.
- Correlate absence/presence of taste and odor species with any taste and/or odor event.

Operational costs

The costs associated with a sampling program will vary dependant on methods used, the volume of sampling required, and geographic differences in personnel costs. However, some aspects of cost can be quantified, particularly capital equipment.

Epifluorescence microscopy equipment costs

Fluorescence microscopes are like all optical equipment; they vary dramatically in price. Entry level scopes of this type retail in the low thousands whereas the microscope used by the Neuer laboratory retails for over $22000 (source: Fischer Scientific). Clearly, product choice for a program of this nature would be influenced by the technical requirements and the funds available.

Semi-automated particle analysis equipment costs

At the time of writing (December 2009) the FlowCAM, and presumably other similar products, ranges in price from $55000 to $85000, dependant on configuration (source: Fluid Imaging Technologies). The annual operating costs are approximately $300, attributable to replacement flow cells and other consumables.

MIB/geosmin analysis

Several commercial facilities offer this type of analysis with a relatively short turnaround time (3-5 working days). The cost varies based on individual customer requirements. Alternatively, in house analysis can be conducted, but the necessary equipment would require a substantial upfront capital investment which could, of course, be redeemed over the life of the program.

Summary

For organizations that are frequently impacted by taste and odor issues, monitoring the source water supply on a regular basis may provide early warning of taste and odor events. By tracking changes in the algal population we have the opportunity to identify the peak bloom conditions that appear to act as a precursor for taste and odor events. The necessary information can be obtained by either the use of traditional sampling, filtering and cell counts using microscopy, or by utilizing an automated technology such as the FlowCAM particle analyzer.

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