1. Scope and Application

1.1 The purpose of this document is to provide a simple set of procedures that can be conducted by designated personnel at a drinking water utility to determine the amount of cyanotoxin that may be removed by powdered activated carbon (PAC) at a drinking water plant.

1.2 PAC is sometimes added to raw water for the removal of organic constituents such as taste and odor producing compounds or for use in managing chemical spill events in the water supply. Some sources of PAC may also be useful for the control of cyanotoxins such as cylindrospermopsin and microcystin-LR. However, removal of the cyanotoxins is affected by the chemical and physical characteristics of the sample, temperature, pH, contact time, carbon type, and PAC dose. The protocol in combination with the PAC Calculator can be used to develop operational charts of dose versus the percent remaining for the target compound at varied conditions and PACs. The following pages detail the protocols for preparing standardized stock solutions, conducting jar tests, and evaluating cyanotoxin removal by PAC.

1.3 This document does not provide protocols for addressing the release of intracellular cyanotoxins that may occur when cyanobacterial cells are oxidized or damaged during other stages of treatment. This protocol ONLY addresses extracellular (dissolved) toxins. Future revisions or additional testing protocols may include recommendations on handling cell lysis and subsequent release of additional cyanotoxins into the water.

1.4 This document was prepared by Ben Stanford, Allison Reinert, and Elisa Arevalo of Hazen and Sawyer, and Craig Adams of Utah State University. This document has been reviewed by the AWWA CCL / Potential Contaminant Technical Advisory Workgroup (TAW), with special thanks to David Cornwell, Keith Cartnick, Rick Sakaji, Issam Najm, and Steve Via for extensive review and comments on testing procedures.

1.5 AWWA makes no guarantee as to the accuracy or applicability of these procedures for a specific utility’s drinking water source or the full-scale applicability of results. The user accepts all liability for use of the procedures described herein and the user is solely responsible for interpretation and implementation of any results derived from the use of these procedures.

1.6 NO PART OF THIS DOCUMENT MAY BE MODIFIED, COPIED, OR DISTRIBUTED WITHOUT PRIOR WRITTEN AUTHORIZATION FROM AWWA. THE USER ACCEPTS LIABILITY ASSOCIATED WITH THE USE OF THE PROCEDURES AND ACCOMPANYING WORKSHEETS AND CALCULATORS.

2. Special Considerations & Initial QA/QC

2.1 Selection of number of samples in jar testing

- All experiments are subject to error, and it is critical that an estimate of this error is included in the testing protocol. This is achieved by running at least some percentage
(e.g., 20%) of the samples in duplicates so that the experimental precision (measured by Relative Standard Deviation (RSD in percent) can be tested.

- If only three or four PAC dosages are of interest to the user, then the number of samples tested could be limited to six (two zero PAC dose samples (duplicate) plus four additional dosages).
- However, to develop more comprehensive and useful operational curves, it is recommended that up to 12 samples be run in total (duplicates of the zero PAC dosage and one other dosage; plus up to eight other dosages).
- This can be conducted on two six-gang stirrers simultaneously (recommended) or in two sequential runs on a single six-gang stirrer (acceptable).
- The associated PAC Calculator is designed to allow a minimum of six samples (with a 0 mg/L (zero) PAC dosage duplicate) and up to 12 total samples.

2.2 Selection of test water will impact the results

- Each water treatment plant is unique in the combinations of source water type/location, treatment processes, chemical usage, etc. When conducting cyanotoxin testing, begin by answer the following question:

  “At which point in the process would you like to determine the impact of PAC on cyanotoxin removal?”

- If the answer is “raw water”, then the test can be conducted with samples of raw water, with the important caveats listed below.
- If the answer is at any other step in the treatment process, then you will need to determine whether to collect water from that point in the process at the plant or whether to mimic the plant’s treatment processes (e.g., floc/sed/filtration, pH adjustment) via jar tests, and then use the water produced at bench-scale to conduct further testing.
- The presence of cyanobacterial (blue-green algae) cells may impact your test results. These procedures are NOT designed to account for cell lysis (i.e., breaking open the cells and releasing additional cyanotoxins into solution). However, PAC is often added in raw water where cyanobacterial cells may be present. Therefore, careful consideration should be given to the following:
  - If you want to quantify the amount of dissolved, extracellular cyanotoxin removed by PAC, this should be done before an actual bloom event occurs to minimize the likelihood of cell lysis biasing the results. In this case you would need to spike cyanotoxins into the raw water solution.
  - If you want to mimic the treatment process, including the impact that damaged or lysing cells will have on cyanotoxin concentration when treated with PAC, then the tests should be conducted during a bloom event when cells are present in the supply. However, you may still need to spike cyanotoxins into the solution to ensure sufficient concentration (100 to 1000 µg/L) to obtain measurable removal results.

- Your test water must have measurable concentrations of cyanotoxins present normally with at least 10 or 20 times the method detection limit. This is important
because it will impact the decision of whether or not you need to spike cyanotoxins into your test water or not. If there is not a sufficient concentration present, you may need to add additional cyanotoxins from a stock solution to achieve the desired initial concentration (e.g., the expected worst-case concentration that can occur during an event, or some other anticipated concentration for an event). While cyanotoxins have been measured in source waters at greater than 20,000 µg/L, most raw water concentrations are less than 1000 µg/L.

- The dose versus percent cyanotoxin remaining curves produced by this protocol and associated PAC Calculator are relatively independent of the initial concentration used provided the initial concentration is sufficiently low (Knappe et al., 1998). HOWEVER, it is strongly recommended that the initial concentration be in the range of anticipated influent (raw) water concentrations expected. (Note: Isotherms calculated from an identical data set are dependent on initial concentration of the adsorbate.)

2.3 Use a composite sample as the starting solution when possible.

- If you are conducting multiple decay tests, collect the entire volume of water needed into a large container or carboy, and use that as the source for each subsequent test.
- If this step is skipped (e.g., if you are collecting multiple jars from a running sample tap), you will need to measure the starting concentration for each separate jar test rather than simply measuring the concentration of cyanotoxin in the large-volume container.

2.4 Maintain a no-treatment “Control” solution during testing

- Two samples (duplicates) from the composite container should be included in the jar testing without any PAC added for the duration of the longest test period.
- Measure the starting concentration in addition to the final concentration of the PAC-free control solution (ensure the cyanotoxin sample collected from the Control is filtered with the same type of filter paper as the test solution samples [see section 5.14]) at the end of the test to determine if there may be some change in cyanotoxin concentration that is not due to the PAC being added to the other jars.
- Little removal without PAC should be observed, and the duplicates should be reproducible (and provide an estimate RSD% of the experiments). The average of the initial cyanotoxin concentration are used in the percent remaining calculations.

2.5 The addition of PAC to the water may impact water quality and treatment

- Addition of PAC to the water may increase the pH and alkalinity of the water which may affect overall coagulation efficiency.
- Jar testing along with ancillary data collection is completed to ensure that effects of PAC on water quality are known prior to full-scale dosing.

2.6 The selection of analytical methods (e.g., ELISA or LC/MS-MS) may impact results
• The current EPA guidance recommends the use of enzyme-linked immunosorbent assay (ELISA) test kits for microcystin-LR and cylindrospermopsin.
• However, it is not fully understood how well those test kits will measure the oxidation (removal) of the cyanotoxins. On the other hand, liquid chromatography with tandem mass spectrometry (LC/MS-MS) will provide more accurate quantitation of the specific cyanotoxins (e.g., MC-LR, MC-RR, etc.) and its subsequent removal, but may not match ELISA test results.
• Therefore, it is recommended that the utility select whichever method will be used to make decisions regarding public notification as the default test method for the procedures documented in this memo.

3. Background Information and Data Needs

3.1 **Powdered activated carbon (PAC)** is typically used to help control periodic taste and odor events and as an emergency treatment for petroleum compounds and pesticides. For this particular protocol, the testing procedures focus on analyzing various PAC doses and types for the removal of cyanotoxins resulting from the presence of cyanobacteria.

3.2 **Bench-scale testing is to be conducted on site at the drinking water treatment plant.**

• A Phipps and Bird (or similar) 6-gang mixer, with 2-liter square jars is recommended to evaluate the impact of PAC on coagulation, flocculation and settling.
• (Other size and shapes of jars require calculational adjustment and are not recommended.)
• Plant staff should conduct bench-scale jar testing on a routine basis and the bench-scale test procedures they use shall be modified when changes in plant flow rate and/or flocculation speeds occur.
• Data collected from the full-scale plant to establish this calibration include:
  o Chemical dosages and application points
  o Plant flow rates
  o Basin surface areas and volumes
  o Mixing intensity (G, sec⁻¹) if known

3.3 **Plant Matching**

• For bench-scale testing, bench-scale rapid mix time is equal to full-scale rapid mix time.
• Bench-scale flocculation time is equal to full-scale flocculation time.
• Jar test rapid mix and flocculation energies can be found by trial and error, or from mixing design criteria provided in engineering reports that stipulates velocity gradients (G) for each process.
• The bench test velocity gradients should match the energy inputs in the full-scale plant (instructions provided in the detailed methodology section).
• The user must convert the full-scale basin loading rate to jar-test settling rate. The equations used to convert the full-scale basin loading rate (X gpd/ft²) to settling rate in the 2-liter jars to establish the appropriate settling time are:

4.1 If cyanotoxins are known to be present in the source water being evaluated, collect a single carboy of water and conduct split sampling from that carboy. If the cyanotoxin concentration or cyanobacteria concentration is unknown in the source water to be analyzed, cyanobacteria or the target cyanotoxin, at a known concentration, shall be spiked into collected source water. The spiked water will then be used in the jars in order to assess the effectiveness of various PAC doses.

4.2 Instructions for preparing and analyzing the cyanotoxin stock and resultant jar concentrations are shown below:

- Collect water to be used for testing, either from bench-top jar tests (note: procedures not presented in this document) or from water collected from within the treatment plant or source, and store in a container with enough volume to accommodate all of the testing that will be completed.
- If the source water does not have the cyanotoxin of interest present, use a commercially-available stock standard (generally available at 10 µg/mL in water) to spike into the water. (IMPORTANT: Cyanotoxin stock standards are toxic by definition, and must only be handled by trained personnel using proper procedures and protocols.)
- Use the following equation to calculate the volume (V) of stock standard needed to spike into the sample water to achieve the desired concentration (C; recommending somewhere between 100 µg/L and 1000 µg/L):

\[
V_{Stock\ Standard} = \frac{C_{Desired\ in\ Water} \times V_{Sample\ Water}}{C_{Stock\ Standard}}
\]

4.3 PAC Wet (Slurry) Dosing Method:
- PAC stock is created in a 10 mg/mL concentration (5 g PAC into 500 mL DI water in flask and stirred continuously on a stir plate).
- PAC should be dosed via a disposable pipette; a plastic Luer Lock syringe; or other appropriate volumetric device with sufficient orifice diameter so as not to get clogged by PAC). Addition should be in the same order it would be added at full-scale (e.g., raw water line, rapid mix, etc.) and with use of associated mixing energies (G values).
- The PAC jar tests for PAC dose should coincide with current chemical additions (e.g., coagulants and polymers, etc.) in order to gauge process changes.
- Recommended PAC doses to evaluate for the removal of cyanotoxins include up to 70 mg/L PAC. The associated PAC Calculator suggests specific dosages to develop operational charts. For example, suggested dosages for a maximum of 50 mg/L PAC might be: 0 (plus duplicate), 1, 2, 4, 7, 10, 15, 22 (plus duplicate), 33, and 50 mg/L. The lower doses are common for taste and odor compounds and other applications, and help provide a “smooth” percent removal versus dose curve from the experimental results.

4.4 **PAC Dry Dosing Method:**
- It is difficult to weigh small amounts of PAC with accuracy. Therefore, the Slurry Method is recommended especially if the lower dosages will be included.
- For laboratories and utilities interested in dosing dry powder, PAC is measured as dry weight into a weighing dish or on balance paper at the appropriate mass (e.g. 20 mg for a 2 L sample to achieve 10 mg/L) and then poured directly into the jar test unit while mixing. It is important that the microbalance used is properly calibrated and measurements are made very carefully.
- Proceed with the remaining instructions from Section 4.3 and 4.5

4.5 During bench-scale testing, samples are collected from all test jars (from the sample taps at the appropriate times) and analyzed for cyanotoxin concentration (via ELISA method, LC/MS/MS method or other method), pH, turbidity, alkalinity and UV-absorbance at 254 nm (UV-254).
- The UV-254 measurement and settled water turbidity are used to determine the process impacts from the use of PAC.
- The volume of sample set aside for UV-254 measurement needs to be filtered prior to analysis with a 0.45-µm nylon or glass fiber syringe filter.

5. **Methodology Part B: Jar Testing** using the AWWA’s Cyanotoxin PAC Calculator.
- The following process should be used to conduct the necessary jar tests

5.1 Obtain six-gang stirrer (e.g., Phipps and Bird Jar Testing Unit).
5.2 Enter number of dosages desired between six and 12 (recommended) in Cyanotoxin PAC Calculator and maximum PAC dosage to be used.

5.3 Based on the suggested dosages by the Cyanotoxin PAC Calculator, enter in the calculator the desired PAC dosages.

5.4 Procure all chemicals that are used at the treatment plant including coagulants, coagulant aids (polymer), and any pH adjustment chemicals (e.g., lime) and prepare the syringes for each jar.

5.5 Prepare PAC stock solution per instructions above and prepare a syringe for dosing PAC.

5.6 Collect water to be tested. This water should be raw water.

5.7 Fill test jars with water to the 2-L fill line.

5.8 Place jars underneath stir paddles in the Phipps and Bird jar tester.

5.9 With the prepped syringes, start the jar tester at the G value for rapid mix and add any associated chemicals that are typically added during rapid mix to the jars.

5.10 Immediately add selected PAC dose for each jar at this time.

5.11 Allow jars to mix at rapid mix speed for equivalent time as plant (plant matching). (DO NOT OVERMIX.)

5.12 Adjust mixing speed (G values) to simulate flocculation of treatment plant and allow to mix at this speed for equivalent time as plant. (DO NOT OVERMIX.)

- Floc formation and PAC contact will be occurring during this time

5.13 Adjust mixing speed (G values) to simulate settling conditions (either very slow or possibly turning off rotation depending on plant conditions). This will vary depending on the type
of flocculation/sedimentation processes present in the plant (e.g., super pulsators, plates and tubes, etc.) and allow to settle for equivalent time as plant.

5.14 Carefully draw sample from sample port on beaker without disturbing settled solids for analysis of cyanotoxin concentration, pH, turbidity, alkalinity and UV254.
- It is recommended that samples for cyanotoxin analysis should be filtered through a 1.2-µm glass fiber filter or equivalent prior to shipping and analysis to remove any remaining high molecular weight organics and particles that may continue to sorb cyanotoxins from the aqueous phase.

5.15 Empty and rinse jars after all samples have been collected and recorded or prepared for shipment to external laboratory for analysis

5.16 After cyanotoxin concentrations have been determined by laboratory analysis, enter results in Cyanotoxin PAC Calculator to analyze results.

5.17 Re-run jar tests with PAC doses that performed the best for cyanotoxin concentration reduction (Steps 5.3 – 5.16):
- Try varying doses of coagulant in the jars with the optimum PAC dose.
- Try varying doses of polymer in the jars with the optimum PAC dose (if applicable).

5.18 Collect samples from second set of jar tests (Step 5.12) and record results.

5.19 Store results for optimum coagulant and polymer (if applicable) doses with best PAC dose for cyanotoxin removal with other standard operating procedures (SOPs) for quick reference during algal blooms or toxin events.

5.20 An example of a datasheet used to organize the data collected from the jar test is shown on the final pages of this document or data can be entered into the PAC Calculator spreadsheet.
6. Analysis and Conclusions

6.1 Record data and information related to the PAC experiments in a laboratory notebook. A suggested method for organizing data is shown in the table on page 10 of this document. Alternatively, the Excel®-based spreadsheet calculator may be used to record and analyze results.

6.2 Upon receipt of the analytical results of the cyanotoxin concentration measurements, record those data in the notebook and spreadsheet as well. Make sure that a hard copy of all laboratory data are stored in the laboratory notebook or binder.

6.3 Confer with management and operations staff to discuss the following:
- **PAC Dose**: Was there a PAC dose that provided the required level of cyanotoxin removal for a given set of water quality conditions?
- **PAC Contact Time**: Does there appear to be sufficient contact time at the proposed PAC dosing location to provide the required removal based on the dose(s) tested?
- **Unintended consequences**: Does the use of PAC change the operation of the other unit processes at the plant? Would the use of PAC require pH adjustment, changes in coagulant dose, or changes in filter operation and backwash? Is the plant able to handle and dispose of the residuals produced during PAC dosing? (What is the plan for residuals disposal?)

6.4 Based on discussions and evaluation of options and unintended consequences, list recommendations on the report form and in the laboratory notebook.

6.5 Place a copy of the test report and recommendations in the test binder stored in the laboratory and provide a copy to the operations manager and all other required personnel.

6.5 This information can be part of a cyanobacterial bloom action plan that should complement other source water management, withdrawal, and treatment modifications.

7. Quality Control

7.1 Any working dilutions of cyanotoxins should be made fresh daily and disposed of in accordance with local regulations or law (potentially, simply down the drain).

7.2 Raw water (or test water) should be collected and used within 24 hours, preferably on the same day of collection. However, allow the solution to reach room temperature or the desired testing temperature (using a water bath) prior to commencing oxidation experiments.

7.4 Dispose of remaining test solution in accordance with local regulations or law (potentially, simply down the drain).

7.5 Wash all glassware immediately after testing is complete.

7.6 Work on clean laboratory surfaces with fresh laboratory bench paper or bench mats.

7.7 Use proper personal protective equipment; use clean gloves to avoid cross-contaminating equipment, surfaces, and glassware.
8. Sources of Additional Information

8.1 AWWA Standard Methods for the Examination of Water and Wastewater, 20th Edition
8.2 Phipps and Bird Jar Testing Procedures
8.3 The UC Center for Laboratory Safety
# JAR TEST STUDY

**Date:** 6/26/2015

**Raw Water Source:** ABC River

**Objective of Study:** To evaluate cyanotoxin removal with various PAC doses

## Plant Match

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**Notes:**

## Optimized PAC Dose

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