Standard Operating Procedure (SOP) 3.4.1.4

By Erick Burres **Updated 3/2011**

Measuring Optic Brighteners in Ambient Water Samples Using a Fluorometer

Introduction

Using Optic Brighteners as Indicators of Wastewater?

Optical brighteners (also known as OBs or OBAs), or fluorescent whitening agents (FWAs in the detergent industry), are compounds that are excited (activated) by wavelengths of light in the near-ultraviolet (UV) range (360 to 365 nm) and then emit light in the blue range (400 to 440 nm). Electrons in fluorescent molecules are excited into a higher energy state by absorption of light and then emit a small amount of heat plus fluorescence as the electrons return to their ground state. Usually, the fluorescence from the second excited state is measured as this can be accomplished with a variety of different pieces of equipment called fluorometers.

Optical brighteners are primarily added to laundry soaps, detergents, and cleaning agents for the purpose of brightening fabrics and/or surfaces. Optical brighteners are dyes that are added to essentially all laundry detergents. These brighteners are adsorbed by fabric and brighten clothing.

Laundry wastewater is the largest contributor of optical brighteners to wastewater systems because it retains a large portion of dissolved optical brighteners. Laundry effluent is predominantly associated with sanitary wastewater. Toilet papers contain fluorescent whitening agents. As toilet paper breaks down, fluorescent whitening agents are released into water. Since optical brighteners decompose relatively slowly except through photo-decay, they serve as ideal indicators (surrogates) of illicit discharges in storm drains, leaking pipes from community wastewater treatment systems, and/or failing septic tanks.

Using optical brighteners as indicators (surrogates) for detecting wastewater has several advantages. Detection is nearly instantaneous, the equipment used is relatively inexpensive, no formal training is needed, and large numbers of samples can be analyzed in a short period of time. It is even possible to conduct “laboratory” operations “in the field”. Where fecal contamination is known or is suspected to occur, the detection of optical brighteners can assist in pollution screening and source identification.

Preparations and Procedures:

Supplies:

Sample Bottles
Foil
Disposable polymethacrylate cuvettes
Permanent marker
Fluorometer (such as an Aquaflor)
UV Lamp (300-400nm excitation & 436nm emission filters; (6W, 365 nm typically used)
UV-proof safety glasses
Stopwatch
Calculator
DI Water
Laboratory Notepad/Datasheets
Calibration standard solution
- Equipment necessary to prepare a calibration solution
  - OB Agent (Tide 2X Original Scent is suggested. Since at least March 2011, all Tide detergents are 2X)
  - Pipette (Piston type)
  - Pipette Tip(s)
  - DI Water
  - 1 liter Erlenmeyer flask & aluminum foil or a 1 liter amber bottle
  - Falcon tube (50ml) or equivalent
  - Tissue -optic brightener free (This can be checked by placing the tissue under the UV lamp and checking for florescence.)

Secondary Standard (optional)
Computer & software: Internal data logging & downloading to a spreadsheet (optional)

Sample Storage:

Fig. 1 Sample wrapped in foil.

The sample must be stored at room temperature and in a lightproof container. An amber bottle or a sample bottle covered with foil can be used (Figure 1).

Always protect the sample from light exposure. Optic brighteners photodecay.

Positioning the Sample (Labeling and Marking the Curvette):

The curvette (Figure 2) needs to be placed in the sample compartment with the same orientation for each measurement taken. Mark the curvette at the top on one side so that the curvette can be placed into the sample compartment the same way each time (Figure 3).

Fig. 2 Disposable polymethacrylate cuvette

Fig. 3 Curvette labeled for positioning

Sample Handling:

Use a clean (new) curvette for each sample. The curvette must be dry on the outside. If it is not possible to use a new curvette for each sample, after cleaning the curvette fill it with a blank solution (DI water) and take a measurement to check for contamination. If the curvette is contaminated do not use it again.

Do not take a measurement if there are air bubbles in the curvette. Remove any bubbles present by lightly tapping on the outside of the curvette wall with your finger, or slightly tilt the curvette to dissipate the bubbles.

Calibration:

<u>Read and follow the instructions for your fluorometer.</u> It is suggested that you use an optic brightener (OB) calibration solution. If this is not available, one can be prepared using a clothes washing detergent such as Tide. If you are using a fluorometer provided on loan by the Clean Water Team, a preset adjustable secondary standard will be provided which will allow the operator to quickly and easily check the fluorometer's calibration stability. If the meter's reading is more that +/-10% of the secondary standard's value, the fluorometer should be recalibrated. Be sure that the calibration value for the 50ppm standard is set to 100 relative fluorescence units (RFU) such as 2 RFU relative to 1ppm of calibration solution (Tide 2x or equivalent).

Preparing a 50ppm OB calibration solution using a clothes washing detergent:

As it can be very difficult to made a 50ppm calibration solution directly, because it requires adding 5ul of detergent (Tide 2X) into 100ml DI it is recommended that a two step serial dilution process be used..

- Prepare a 1 liter Erlenmeyer flask covered with aluminum foil to make it light-proof or a 1 liter amber bottle with 100 ml of DI water.

- Using a piston style pipette, draw 0.5ml of OB agent (Tide 2X Original Scent is suggested). Wipe off excess OB agent that might have coated the pipette tip. Dispense the OB agent into the 1 liter vessel of DI water, cap and mix thoroughly. Allow foam to settle before next step*. (This solution is 500ppm Tide 2X and can be reserved as <u>stock</u> for further use.)

- To then make the actual calibration solution (50.0ppm Tide 2X), add 5.0ml of the stock solution to 45ml of DI water in a foil wrapped Falcon tube. Cap the tube and mix thoroughly. Allow foam to settle before use*.

- It may take quite a long time for foam to settle

Fig. 4 Fluorometer

Fig. 5 Secondary Standard

Label 3 disposable polymethacrylate cuvettes per sample (Analyze triplicates for each sample).

Load 3mls of sample into each curvette (protect the sample from light as much as possible during loading). If 3mls of sample is not available be sure that at least 2ml of sample is used (1/2 of the curvette is full).

Assigning a Calibration Standard Value (Aquafluor):

1. Press the <STD VAL> button.
2. Use the - and + arrow buttons to set the standard value. Holding down either arrow button down will allow you to change the value using fast scrolling.
3. When finished, Press the <ENT> or <ESC> button to accept the value and to return to the Home screen.

Performing the Calibration (Aquafluor):

1. Press the <CAL> button.
2. Press <ENT> to start the calibration.
3. Insert your blank sample and press <ENT>. The Aquafluor will average the reading for 10 seconds and set the blanking zero point.
4. Insert the standard sample and press <ENT>. The reading is averaged for 10 seconds and the Standard Calibration value is set.
5. Press <ENT> when the calibration is complete to accept the calibration. If <ENT> is not pressed within 10 seconds, you will be asked if you want to abort the calibration. Aquafluor™ User's Manual 12 Press the ↑ or ↓ arrow button to abort or accept the calibration respectively. If at anytime during steps 1-4 you want to stop the calibration, press <ESC>. This will return you to the Home screen and will default the instrument to the previous calibration.

Sample Preparation:

Label 3 disposable polymethacrylate cuvettes per sample (Analyze triplicates for each sample).

Load 3mls of sample into each cuvette (protect the sample from light as much as possible during loading). If 3mls of sample is not available be sure that at least 2ml of sample is used (1/2 of the cuvette is full).

Sample Analysis:

Turn the fluorometer on.

Insert the sample.

Press the <READ> button.

The reading result will appear on the top line of the Home screen.

Once the word "WAIT" disappears from the Home screen another reading can be made. During each reading the sample is warmed.

Be sure that you wait for each sample to equilibrate to room temperature before each reading is made.

Analytical Procedure:

As the analytical procedure is followed, ensure that all sample information and data is recorded as it relates to the analytical decision process (Table 1). Examples of laboratory data sheets can be found in Appendix A and B.

Step 1.
Measure initial fluorescence using Aquafluor (or equivalent)

If the sample measures <5ppm conclude that the sample is negative for optical brighteners.

If the sample measures higher than >5ppm, continue to step 2.

Step 2.
Expose samples directly to UV light for 5minutes and then measure fluorescence again. Calculate the percentage of reduction in fluorescence after 5min compared to before UV exposure

If % reduction < 8%, conclude the sample is negative for optical brighteners.

If % reduction ≥30%, conclude the sample is positive for optical brighteners.

If % reduction <30% and >8%, continue to Step 3.

Step 3.
Expose samples under UV for another 5min (i.e. accumulatively 10min), measure fluorescence, calculate the ratio of % reduction in fluorescence after 10min UV exposure over % reduction after 5min UV exposure

If the ratio is no less than (equal to or greater than) 1.5, conclude that the sample is negative for optical brighteners.

If the ratio is less than 1.5, conclude that the sample is positive for optical brighteners.

Step 4.
Out of the 3 replicates

If all three are positive → conclude that the sample is positive for optical brighteners.

If two out of three positive → conclude that the presence of optical brighteners within the sample is undetermined.

Fig. 6 Prepared (spiked) sample under UV light exposure

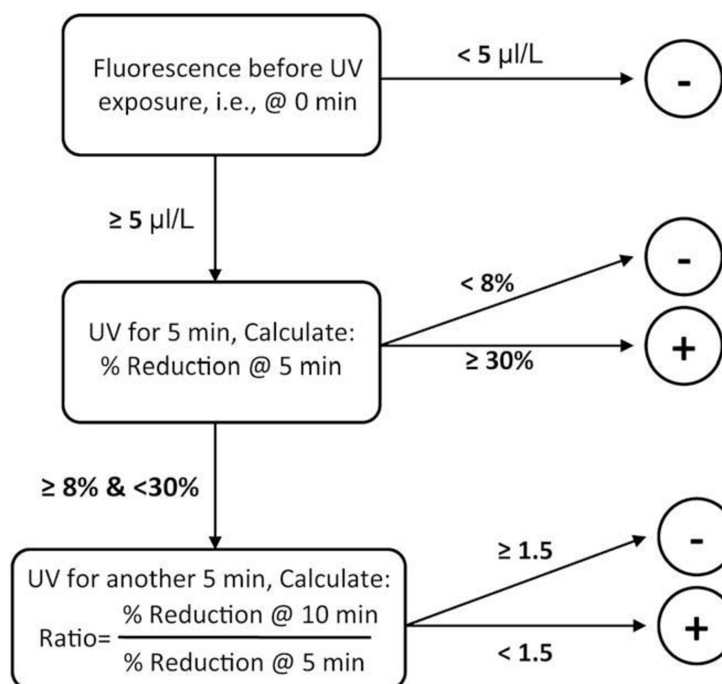

Diagram 1. Analytical Procedure Decision Tree (Cao, 2008)

Sample ID	Date	Replicate	Fluoresc. at 0 min	Step1	Fluoresc. at 5 min	%Reduction in fluoresc.at 5 min	Step2	Fluoresc. at 10 min	%Reduction in fluoresc. at 10 min	Ratio of %reduction at 10min to that at 5min	Step3	OB Result	Notes	QA-1	QA-2	SampleID	Date	Avg. Initial fluorescence (RFU)	Presence or Absence of OB
Example	3/17/09	1	30	UV	15	50	Positive		n/a	n/a	n/a	Positive		1	1	Example	3/17/2009	21.33	Undetermined
		2	25	UV	20	20	UV	18	28.0	1.4	Positive	Positive		1	1				
		3	9	Negative		n/a	n/a		n/a	n/a	n/a	Negative		0	0				
Legend																			
	Cells that will be filled during the measurement																		
	Cells that give results (Positive, Negative) or instruction (UV) for next step																		
	Lab results on OB detection (Positive, Negative)																		
	Final results to report																		
QA-1,QA-2	*QA: the two columns should completely agree with each other*																		

Table 1. Example of a Laboratory Data Sheet

OPTIONAL

Internal Data Logging and Data Downloading to a Spreadsheet (Example using software for the Aquafluor):

Activate internal data logging:

Press the <DATA> button 2 times.
Press the <ENT> button to toggle between logging and stop status.
Press the <ESC> button when finished to return to the Home Screen.

Download data:

Connect the fluorometer to the serial port of your computer.
Open the software (Spreadsheet Interface Software- SIS for 380).
< Follow manufacturer instructions to install software.>

Press the <DATA> button 3 times.

Press the <ENT> button 5 times to start the data download.
Press the <ESC> button when finished to return to the Home Screen.

Erase data:
Press the <DATA> button 4 times.
Press the <ENT> button 5 times to erase all logged data.
Press the <ESC> button when finished to return to the Home Screen.

Deactivate internal data logging:
Press the <DATA> button 2 times.
Press the <ENT> button to toggle between logging and stop status.

Fig. 7 Software and data cable

Appendix A Example of a Laboratory Data Sheet (Cao 2009)

Sample ID	Date	Initial fluorescence (RFU)	Presence or Absence of OB

Appendix B Laboratory Data Sheet Example From Table 1 (Cao 2009)

Sample ID	Date	Replicate	Fluoresc. at 0 min	Step1	Fluoresc. at 5 min	%Reduction in fluoresc.at 5 min	Step2	Fluoresc. at 10 min	%Reduction in fluoresc. at 10 min	Ratio of %reduction at 10min to that at 5min	Step3	OB Result	Notes	QA-1	QA-2	SampleID	Date	Avg. Initial fluorescence (RFU)	Presence or Absence of OB
Example	3/17/09	1	30	UV	15	50	Positive		n/a	n/a	n/a	Positive		1	1	Example	3/17/2009	21.33	Undetermined
		2	25	UV	20	20	UV	18	28.0	1.4	Positive	Positive		1	1				
		3	9	Negative		n/a	n/a		n/a	n/a	n/a	Negative		0	0				

Legend

(grey)	*Cells that will be filled during the measurement*
(purple)	*Cells that give results (Positive, Negative) or instruction (UV) for next step*
(yellow)	*Lab results on OB detection (Positive, Negative)*
(teal)	*Final results to report*
QA-1,QA-2	*QA: the two columns should completely agree with each other*

References

Cao, Yiping and John F. Griffith, Stephen B. Weisberg. 2009. Evaluation of optical brightener photodecay characteristics for detection of human fecal contamination. Water Research http://www.elsevier.com/locate/watres

Ciba Specialty Chemicals, Inc. 2004. Human and environmental risk assessment on ingredients of European household cleaning products: Fluorescent Brightener FWA-1. http://www.heraproject.com/RiskAssessment.cfm

Ciba Specialty Chemicals, Inc. 2004. Human and environmental risk assessment on ingredients of European household cleaning products: Fluorescent Brightener FWA-5. http://www.heraproject.com/RiskAssessment.cfm

Close, M. E., L. R. Hodgson, and G. Todd. 1989. Field evaluation of fluorescent whitening agents and sodium tripolyphosphate as indicators of septic tank contamination in domestic wells. New Zealand Journal of Marine and Freshwater Research 23:563-568.

Gilpin, B. J., and D. Saunders. 2005. Fluorescent whiteners as indicators of human effluent. Environmental Detection News 2:1-2 and 6-7.

Gilpin, B. J., J. E. Gregor, and M. G. Savill. 2002. Identification of the source of fecal pollution in contaminated rivers. Water Science and Technology 46:9-15.
Poiger, T., J. A. Field, T. M. Field, H. Siegrist, and W. Giger. 1998. Behavior of fluorescent whitening agents during sewage treatment. Water Research 32:1939-1947.

Hagedorn, C., R. B. Reneau, Jr., M. Saluta, and A. Chapman. 2003. Impact of onsite wastewater systems on water quality in coastal regions. 23pp. Final Project Report to the Virginia Department of Health and the Virginia Department of Conservation and Recreation, Richmond, VA. Project No. 50312-01-13-PT.

Hagedorn, C., R. B. Reneau, Jr., M. Saluta, and A. Hassall. 2005.Fluorometric Detection of Optical Brighteners as an Indicator of Human Sources of Water Pollution. Part I. Description and Detection of Optical Brighteners. Crop and Soil Environmental News, November 2005

Hagedorn, C., R. B. Reneau, Jr., M. Saluta, and A. Hassall. 2005.Fluorometric Detection of Optical Brighteners as an Indicator of Human Sources of Water Pollution. Part II Development as a Source Tracking Methodology in Open Waters Crop and Soil Environmental News, November 2005

Hayashi, Y., S. Managaki, and H. Takada. 2002. Fluorescent whitening agents in Tokyo Bay and adjacent rivers: Their application as anthropogenic molecular markers in coastal environments. Environmental Science and Technology 36:3556-3563.

Iyer ,Seshadri, M. Barbachem S. McLaughlin, and W. Johnston. 2006. Monitoring Optical Brighteners Helps Track Watershed Pollution. Water World, December 2006 http://w w .pennnet.com/display_article/280471/41/ARTCL/none/none/1/Monitoring-Optical-Brighteners-Helps-Track-Watershed-Pollution

Sargent, D., and W. Castonguay. 1998. Water quality sampling: An optical brightener handbook. http://www.naturecompass.org/8tb/sampling/

Sinton, L.W., R. K. Finlay, and D. J. Hannah. 1998. Distinguishing human from animal faecal contamination in water: a review. New Zealand Journal of Marine and Freshwater Research 32:323-348.

Turner Designs. 2004. Aquafluor: Handheld Fluorometer and Turbidimeter-A User's Manuel. P/N 998-0851

Ullmans Encyclopedia of Industrial Chemistry. 2001 Electronic release, 6th Edition. Wiley-VCH Interscience. http://www.wiley-vch.de/contents/ullmann/ull_10518.html

URS Corporation. 2004. Optical brightener study, Lynnhaven Bay Watershed. 87pp. Draft Report to the City of Virginia Beach, Department of Public Works, Virginia Beach, VA. Project No. 11655695.

Waye, D. 2003. Detecting sewage leaks with optical brightener monitoring. Volunteer Monitor 15:16-17.