Appendix 2. Description of Research Method for Analysis of Optical Brighteners in Water

Optical brighteners have been used in conjunction with bacterial source tracking techniques as indicators of wastewater in a variety of environmental settings (Hayashi and others, 2002; Boving and others, 2004; Hagedorn and others, 2005; Dickerson and other, 2007). Optical brighteners are components of most laundry detergents and function as dyes that fluoresce upon exposure to ultraviolet light. The resulting fluorescence causes white materials to appear less yellow and colors to appear brighter when exposed to sunlight. Because water from laundering of clothing is a component of domestic wastewater, these compounds typically occur in conjunction with fecal wastes. The primary route of degradation for optical brighteners in the environment is exposure to ultraviolet light (Kramer and others, 1996). In darkness, optical brighteners are stable in water and, based on structure, are not expected to undergo hydrolysis (Harris, 1990). Poiger and others (1998) found little biodegradation of optical brighteners during treatment of municipal wastewater. However, about 85 percent of the optical brighteners adsorbed to sludge during the treatment process.

Various analytical techniques have been applied to the detection of optical brighteners ranging from technically demanding methods such as solid-phase extraction and high-performance liquid chromatography (Stoll and Giger, 1997; Shu and Ding, 2005) to simple fluorometry. Although high-performance liquid chromatography (HPLC) methods are sensitive and specific, these methods require expensive equipment and expertise not available to most health departments. As a result, there is much interest in improving fluorometric methods of analysis that require only inexpensive, simple-to-operate equipment that is capable of being used in the field.

One of the major shortcomings of fluorometric analysis of optical brighteners is interferences from natural organic matter (Wolfe, 1995). Hartel and others (2007) found that use of a narrow-band emission filter (436 nanometers, nm) instead of the commonly used wideband filter (410 to 600 nm) reduced fluorescence from background natural organic matter. In addition, Hartel and others (2007) were further able to distinguish fluorescence from natural organic matter from that of optical brighteners by assessing the loss of fluorescence upon exposure to ultraviolet light. The researchers found that preferential photo decay of optical brighteners by a 5-minute exposure period to ultraviolet light resulted in greater loss of fluorescence for samples containing optical brighteners. Thus, they were able to establish that a loss of more than 30 percent of the original fluorescence following exposure to ultraviolet light (while in glass cuvettes) indicated the presence of optical brighteners. Cao and others (2009) found that the method of Hartel and others (2007) failed to accurately identify the presence of optical brighteners in about half of the samples from southern California streams. As a modification to the method of Hartel and others (2007), Cao and others (2009) employed a two-phase ultraviolet exposure approach in which the initial and subsequent rate of loss of fluorescence were compared. Because optical brighteners undergo much more rapid photo decay than natural organic compounds, most of the optical brighteners are degraded within the first 5 minutes of exposure to ultraviolet light. Thus, loss of fluorescence during a second 5-minute period of ultraviolet light exposure results primarily from degradation of natural organic compounds, which are less susceptible to photo decay and typically have linear photo decay curves. As a result, comparison of the rates of fluorescence loss during the first and second exposure periods can indicate the presence of optical brighteners. Because of the need for ultraviolet light chambers, these methods are not easily used in a field setting.

Optical brighteners are effective at dyeing laundry because of their affinity for fabrics. Poiger (1994) estimated that 74 percent of the optical brighteners in detergents were bound to fabrics during a wash cycle. In addition, there is some variation in the affinity of optical brighteners with respect to type of fabric. Cellulose has generally been recognized as having a high affinity for optical brighteners. In particular, cotton pads, which are predominately cellulose, have been used to detect optical brighteners in wastewater. The pads are placed in an area where a source of wastewater is suspected for several days, retrieved, and tested for fluorescence (Hagedorn and others, 2005). This high degree of affinity of optical brighteners for cellulose also provides an opportunity to assess the presence of these compounds in water samples.

The proposed method exploits the affinity of optical brighteners for cellulose and is based on the concept that upon addition of cellulose fibers to a water sample containing optical brighteners, these compounds will preferentially bind to the cellulose fibers. For this method to be effective, the addition of cellulose should not affect the fluorescence of a sample in which optical brighteners are absent. In addition, the length of time required for binding between optical brighteners and cotton fibers must be assessed.

To assess the effects on cellulose of the fluorescence of natural organic compounds, potential sources of natural fluorescence were investigated. Aqueous extracts of senesced and green foliage from vegetation in the North Carolina Piedmont were prepared by soaking 25 grams (g) of the vegetation in 200 milliliters (mL) of deionized water for 72 hours. Fluorescence of the extracts was measured in equivalent concentration units of fluorescent brightener 28 (FB-28) in micrograms per liter (µg/L) (table 1). Extracts from conifers had much higher fluorescence that those from other types of vegetation. Cellulose fiber (0.2 g) was added to a 20 mL volume of the aqueous extracts of foliage from several conifers and rotated for 2 hours to keep the cellulose in suspension. Following rotation, the extracts were centrifuged to remove the cellulose fiber and fluorescence was measured. Exposure to cellulose fibers did not result in decreased fluorescence.

Table 1. Fluorescence of aqueous extracts of foliage from vegetation in the study area.
[Raw extracts were prepared by soaking 25 grams of plant matter in water for 72 hours. Cellulose treatment included addition of cellulose fiber, rotation, and filtration to remove the fiber; --, not analyzed]

	Fluorescence[a]	
Foliage type	Raw	Cellulose- treated
Tsuga canadensis (senesced)	150	150
Pinus taeda (senesced)	82	81
Pinus taeda (green)	64	88
Cupressus leylandii (senesced)	330	320
Juniperus virginiana (green)	310	310
Liquidambar styraciflua (senesced)	8	--
Acer rubra (senesced)	3	--
Quercus alba (senesced)	6	--
Ilex americana (green)	7	--
Magnolia grandiflora (senesced)	11	--

[a]Fluorescence units equivalent to FB-28 concentration in µg/L.

To further assess the effects of cellulose fiber on the fluorescence of natural organic matter, streamwater samples were obtained from several areas in North Carolina, including Big Swamp near Tarheel, Black River near Currie, New River near Gum Branch, and Rhodes Creek tributary near Durham. These streams drain areas in which human activities, and the likelihood of the presence of optical brighteners, is minimal. The addition of cellulose fibers (0.2 g) to 100-mL samples of water from these sites did not affect fluorescence. Thus, it appears that the addition of cellulose is unlikely to affect the fluorescence of natural waters.

The second phase of this study addressed the effectiveness of cellulose fibers in binding optical brighteners and explored use of sodium chloride and a surfactant to facilitate binding. Four sets of spiked samples of natural water were used for this study. Two sets were spiked with FB-28 at a high level (yielding a concentration of about 24 µg/L), and a low level (yielding a concentration of about 12 µg/L) and two sets were spiked with Original Scent Tide® at a high and low level (calculated to yield increases of fluorescence equivalent to a concentration of about 42 µg/L and 21 µg/L, respectively, in FB-28 equivalents). These samples, and unspiked water samples, were treated with cellulose at high (0.4 g per 100 mL) and low (0.2 g per 100 mL) levels; low-level cellulose and sodium chloride, low-level cellulose, sodium chloride, and the surfactant polyoxyethylene lauryl ether. Fluorescence was measured at 2 and 4 hours following the addition of cellulose. Samples were rotated to keep the cellulose fibers in suspension and thereby facilitate interaction with optical brighteners. Samples were allowed to settle prior to filtration with a 0.45-micron syringe filter to remove cellulose fibers.

Results of the experiment are provided in table 2. The low level of cellulose was adequate for removal of all levels of optical brighteners and the addition of high levels of cellulose did not result in a more rapid decrease in fluorescence (fig. 1). Additions of salt and surfactant appeared to have made little change in removal rates. The exception was the low concentration FB-28 samples where fluorescence was lower in samples with added salt and surfactant. Additions of salt have shown to result in slight decreases in fluorescence (table 3), likely the result of “salting out” of poorly soluble organic compounds. The fluorescence of some of the samples to which salt had been added declined to a level slightly less than that of the unspiked water (table 2). Thus, the utility of adding salt appears limited and may compromise the analysis. Likewise, the addition of the nonionic surfactant appears to confer no advantage. Concentrations of optical brighteners, calculated by subtracting the final fluorescence from the initial fluorescence, indicated average recoveries of more than 80 percent. Some of this difference may be the result of matrix interferences or interactions with components of natural water. However, using cellulose to bind optical brighteners appears to be a valid and semi-quantitative approach for estimating the concentration of optical brighteners in water samples.

Table 2. Effects of addition of cellulose, salt, and a surfactant on fluorescence of natural water amended with the optical brightener FB-28.

[µg/L, microgram per liter; g, gram, mL, milliliter; FB-28, Fluorescent Brightener 28]

		Spiked with FB-28 (high level)			Spiked with FB-28 (low level)		
Treatment	**Time (minutes)**	**Fluorescence (as µg/L FB-28)**	**Standard deviation**	**Calculated optical brightener concentration**	**Fluorescence (as µg/L FB-28)**	**Standard deviation**	**Calculated optical brightener concentration**
Cellulose (low level, 0.2 g/100 mL)	0	77.9	0.43		67.9	0.54	
	120	58.2	0.17	20.4	57.6	0.4	10
	240	57.4	0.26		57.9	0.16	
Cellulose (high level, 0.4 g per 100 mL)	0	77.9	0.43		67.9	0.54	
	120	58.3	0.09	20.2	58	0.24	10
	240	57.7	0.37		57.9	0.49	
Cellulose (low level) + Salt	0	77.9	0.43		67.9	0.54	
	120	56.7	0.19	21.5	56.7	0.13	11.4
	240	56.4	0.51		56.5	0.28	
Cellulose (low level) + Salt + Surfactant	0	77.9	0.43		67.9	0.54	
	120	56.7	0.19	21.5	56	0.29	12.2
	240	56.4	0.51		55.8	0.27	

Table 3. Effects of addition of cellulose, salt, and a surfactant on fluorescence of natural water amended with Tide® detergent.

[µg/L, microgram per liter; g, gram, mL, milliliter; FB-28, Fluorescent Brightener 28]

		Spiked with Tide® (high level)			Spiked with Tide® (low level)		
Treatment	**Time (minutes)**	**Fluorescence (as µg/L FB-28)**	**Standard deviation**	**Calculated optical brightener concentration**	**Fluorescence (as µg/L FB-28)**	**Standard deviation**	**Calculated optical brightener concentration**
Cellulose (low level, 0.2 g/100 mL)	0	99	0.15		78	0.23	
	120	58.9	0.04	40.6	58.1	0.15	21
	240	58.4	0.2		57.2	0.23	
Cellulose (high level, 0.4g/100 mL)	0	99	0.15		78	0.23	
	120	59.3	0.47	41	57.9	0.23	20.5
	240	58.4	0.12		57.5	0.38	
Cellulose (low level) + Salt	0	99	0.15		78	0.09	
	120	57.7	0.63	41	57	0.37	21
	240	57.4	0.3		56.8	0.27	
Cellulose (low level) + Salt + Surfactant	0	99	0.15		78	0.23	
	120	56.8	0.23	41	57	0.32	21
	240	57.5	0.49		56.8	0.4	

Method

A Barnstead-Turner Quantech fluorometer equipped with narrow band 360-nm (excitation) and 440-nm (emission) filters was used for fluorometric analysis. A quartz cuvette was used for analyses described in this document. Standards were prepared using Fluorescent Brightener 28 (Chemical Abstracts Service Registry Number, CASRN 4404-43-7) in organic free deionized water. A secondary set of standards was prepared using Original Scent Tide® in organic free deionized water. Standards were prepared on a weekly basis to prevent decomposition and the fluorometer was recalibrated on a daily basis. Cellulose fibers (CASRN 9004-34-6), categorized as medium size, were obtained from Sigma-Aldrich Company. Polyoxyethylene lauryl ether (CASRN 9002-92-0) and sodium chloride were investigated as potential additives to facilitate binding of the fluorescent brightener to cellulose.

A 50-mL water sample was transferred to a polyethylene centrifuge tube. Cellulose fiber (0.2 g) was added to the water and the sample was rotated for 1 hour to keep the cellulose fiber in suspension. After 1 hour, the centrifuge tube was removed from the rotator and the fiber was allowed to settle. Following 1 hour of settling, an aliquot of water was removed from the centrifuge tube and either filtered or centrifuged to remove any cellulose fiber from the sample. Avoid contact with the sides of the centrifuge tube when removing the sample to minimize the cellulose fiber in the sample.

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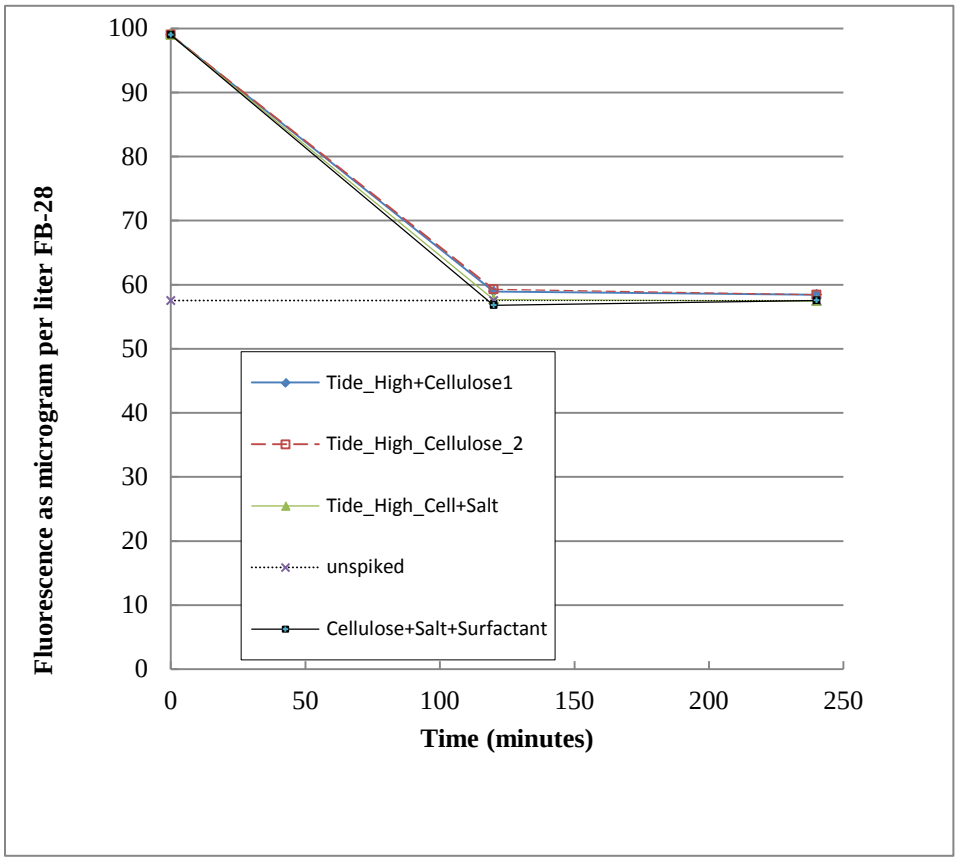

Figure 1. Change in fluorescence over time in samples amended with Tide® detergent, salt, polyoxyethylene lauryl ether, and cellulose.