Evaluation of optical brightener photodecay characteristics for the detection of human fecal contamination

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Abstract

Detection of optical brighteners (OBs) by fluorometry combined with ultraviolet (UV) light exposure has been proposed as an inexpensive method for the detection of human fecal contamination, but this approach has received limited testing. This study evaluated the approach in southern California by applying it to a variety of detergents, sewage, and septage samples from the region, as well as to natural stream water as a negative control. The concept of using UV exposure to differentiate fluorescence from natural organic matter proved valid, as the method produced no false positives. However, the method failed to detect half of the detergents tested in natural stream water at 5 µl/L, due to its conservative thresholds. This study identified a method modification that allows lower thresholds by taking advantage of differences in shape of photodecay curves between OBs and natural organic matter. This method modification resulted in detection of all detergents, sewage at 1:10 dilution and septage at 1:100 dilution. However, several caveats for its use remain, as the OB signal degraded rapidly in strong sunlight. Additionally, low sensitivity for some environmentally-friendly detergents was observed, which does not present a problem on a community basis where a mix of detergents are used, but could be of concern for assessing septic inputs from individual homes.

Introduction

Traditional methods of measuring beach water quality by enumerating fecal indicator bacteria (FIB) do not identify contamination sources, which is often necessary for effective remediation. A broad array of microbial source tracking (MST) methods has been developed to identify sources, including culture-dependent phenotypic and genotypic library-based methods, culture-independent genotypic methods and chemical methods (Simpson *et al*. 2002, Scott *et al*. 2005). However, most of these methods are slow, expensive, and too complex for application in the field or by laboratories that do not have the capability for employing molecular methods.

Measurement of OBs to assess the presence of human waste streams has been suggested as an alternative to these complex methods. OBs are whitening agents added to most modern laundry detergents. The mixing of wastewater and grey water in household plumbing systems allows for the detection of OBs in both septic (Close *et al*. 1989, Boving *et al*. 2004) and sewage systems (Poiger *et al*. 1998). OBs can be measured with great sensitivity using liquid chromatography (Stoll and Giger 1997, Shu and Ding 2005), but measurement of fluorescence is the more frequently used method for source tracking applications (Sargent and Castonguay 1998, Dickerson *et al*. 2007, Hartel *et al*. 2007b). Whereas the former is sensitive, it is also costly, requires advanced operating expertise, and often involves slow, complicated extraction protocols. In contrast, fluorometry is simple, quick, inexpensive, and field friendly.

The concern with OB measurement using fluorometry is that some organic matter and aromatic compounds absorb and emit light at wavelengths similar to that of OBs, potentially confounding the measurements (Boving *et al*. 2004, Hagedorn *et al*. 2005). Hartel *et al*. (2007a) suggested that this confounding can be resolved by combining fluorometry with UV light exposure because OBs degrade rapidly under UV (Kramer *et al*. 1996), while the confounding compounds do not. The change in fluorometric readings before and after UV exposure represents the approximate amount of OBs present in the water sample and can serve as a confirmatory test that the fluorescent compound in the water is due to OBs and not materials that fluoresce under the same conditions (Hartel *et al*. 2007a).

While the Hartel *et al*. method is promising, it was developed and tested only in the southeastern United States. Although it performed well in that

area, there are differences in human wastewater composition and other natural potentially interfering compounds in other regions. This study presents an evaluation of this method in southern California.

METHODS

The use of OBs as a wastewater marker was assessed in three ways. First, the study assessed whether the Hartel *et al*. method successfully identified the presence of OBs in detergents, sewage, and septage. Second, the fluorescence and photodecay rate of natural organic matter was measured for comparison to that of detergents, sewage, and septage. Third, the study assessed how sunlight affects signal stability of the OBs.

Twenty-one detergents were purchased at local markets in southern California and diluted to a concentration of 5 µl/L with natural stream water (NSW) from Malibu Canyon, California. These solutions were then exposed to UV light with fluorometric readings taken at 0, 2, 5, 10, 15, and 30 minutes in polymethacrylate cuvettes (10 x 10 x 45 mm, Turner Designs, Sunnyvale, CA). UV exposure was conducted with a UVP xx-15BLB bench lamp (Ultra Violet Products, Upland, CA) in a dark hood. UV exposure was conducted in an air-conditioned room with a fan to minimize potential heat buildup, since fluorescence can be temperature dependent (Smart *et al*. 1976).

All measurements were obtained using a Turner Designs Model 10-AU equipped with 300 - 400 nm excitation and 436 nm emission filters (Turner Designs, Sunnyvale, CA): these are the same settings used in Hartel *et al*. (2007a). The instrument was calibrated following the instrument manual with the sensitivity set to the medium range. For the calibration, the blank was deionized water (DI) and the standard was 50 µl/L (in DI) of Tide laundry detergent (Tide Original Scent 2X, Proctor and Gamble, Cincinnati, OH), set to a fluorometric value of 100 units. This equivalence of 100 fluorescent units to 50 ppm Tide detergent was then used to convert fluorescence units of samples to Tide detergent equivalent (Tide equivalent) in µl/L. All measurements were obtained in triplicate and reported as averages.

Sewage samples were obtained as primary influent (PI), primary effluent (PE), secondary effluent following activated sludge treatment (SS), and secondary effluent following trickling filter treatment (SF) from the Orange County Sanitation District (OCSD, Fountain Valley, CA). Samples were collected around 9 a.m. for six consecutive days (May 12 - 17, 2007). Primary influent and primary effluent were also collected from the Encina Wastewater Authority (Carlsbad, CA) on July 2, 2007, and from Morehead City Wastewater Treatment Plant (Morehead City, NC) on July 31, 2007. The samples were tested at a 1:10 dilution with NSW. Septage was obtained from a septage delivery truck to the OCSD on March 27, 2007, and tested at dilutions of 1:20 and 1:100 in DI.

Fluorescence degradation of natural organic matter (NOM) was quantified using samples from three stream locations in California watersheds that are believed to be free from human influence: two locations in Escondido Creek and one in Ramirez Creek. In addition, two samples were created by adding Nordic Reservoir Organic Matter (International Humic Substances Society) to DI at the concentrations of 20 and 40 µl/L. The NOM samples were exposed to UV light for up to two hours.

Two experiments were conducted to evaluate stability of the OB signal. To determine whether constituents in sewage or septage consume OBs, sewage and septage samples were amended with a commercial detergent (All Small & Mighty, 10 µl/L) and measured for photodecay before and after a three-hour storage period in the dark, at room temperature. Next, a sunlight exposure experiment was conducted in which 15 detergents were mixed in NSW at a concentration of 5 µl/L and placed into 2 sets of 33 cuvettes: one set exposed in the sun and the other set was placed in shade at the same spot. At 11 time points, triplicate cuvettes were taken from both the in-sun and in-shade sets, moved to an air-conditioned laboratory, and kept in the dark. Fluorescence was measured 40 minutes after the cuvettes had been taken back to the laboratory, allowing the solution to cool to room temperature before initial fluorometric readings. All cuvettes were calibrated with DI prior to the experiment to compensate for possible minor optical difference among cuvettes. Temperature and UV index were recorded using http://weather.com for the zip code 92626 where the experiment was conducted (Costa Mesa, CA). The experiment was repeated at the same location around noon on a sunny day (12:04 p.m. - 4:04 p.m., September 26, 2007), and in the morning on a slightly cloudy day (8:30 a.m. -1:30 p.m., October 3, 2007).

RESULTS

The Hartel *et al*. (2007a) method identified samples as positive for OBs if initial fluorescence was greater than 30 fluorometric units (which equates to 15 µl/L of Tide detergent in the present study's calibration curve) and fluorescence reduced more than 15% when exposed to UV light for 5 minutes. In the present study, none of the negative controls tested positive for OBs. Initial fluorescence for the NSW was low, equivalent to the fluorescence produced by 3 - 7 µl/L of Tide detergent (Table 1). The spiked NOM samples had higher initial fluorescence, ranging as high as 15 µl/L Tide equivalent, but fluorescence for both the NSW and the NOM samples decayed slowly when exposed to UV light, declining less than 2% after five minutes and less than 10%, after two hours of UV exposure (Table 1).

Initial fluorescence values for detergents varied considerably both within and across manufacturers (Table 2). Arm and Hammer Clean Burst had the highest, whereas Biokleen (which contains no OBs) had zero fluorescence even when measured at 50 µl/L. Only 11 of the 21 detergents tested positive for OBs using the Hartel *et al*. method. Of the 10 detergents that tested negative, 8 failed because of initial fluorescence that was less than 15 µl/L Tide equivalent. The photodecay curves of all detergents, except Bi-O-Kleen, were characterized by sharp drops in fluorescence within minutes and subsequent flattening out for the remainder of the UV light exposure. However, for 7 of the detergents, the after-five-minute degradation was less than 15%: the threshold used by Hartel *et al*. (2007a) to distinguish positive from negative. Two detergents that exceeded the initial fluorescence threshold were classified negative because of less than 15% signal degradation after 5 minutes of UV exposure.

Initial fluorescence for sewage was fairly consistent among facilities, at approximately 10 µl/L Tide equivalent (Table 3). Photodecay rates for sewage were also less than those for detergents, with only the influent sample for the OCSD exceeding the 15% degradation threshold after five minutes of UV exposure. None of the sewage samples from any of the sanitation facilities were classified as positive for OBs based on the Hartel *et al*. (2007a) thresholds. However, photodecay curves for all sewage influents were similar to those for detergents and characterized by a sharp drop within minutes of UV exposure. On the other hand, photodecay curves for all secondary effluents were similar to those for linear decay (a straight line with a negative slope; Figure 1).

Initial fluorescence for septage was 45 and 10 µl/L Tide equivalents when diluted 1:20 and 1:100, respectively (Table 3). Photodecay rates for septage were greater than those for either sewage influent or effluent regardless of dilution. The 1:20 dilution sample was classified as positive, while the 1:100 dilution was not because initial fluorescence was too low. The photodecay curves were similar to the graph of a reciprocal function.

The OB fluorescence signal was found to be stable, except when samples were exposed to sunlight. Photodecay curves for both sewage and septage were identical before and after a three-hour storage period

Table 1. Photodecay characteristics of NSW and NOM.

Sample	Initial Fluorescence (µL/L Tide equivalent)	Reduction in Fluorescence after UV Exposure (%)				
		2 min	5 min	10 min	30 min	>120 min
NSW from Upper Escondido Creek	5	7	12	19	24	-
NSW from R2 Creek, Ramirez Canyon	7	2	3	3	5	-
NSW from Lower Escondido Creek	3	0	1	4	21	-
10 µL/L NOM in DI	5	0	0	0	4	7
40 µL/L NOM in DI	15	3	2	3	4	9

Table 2. Photodecay characteristics of a variety of detergent solutions at the concentration of 5 µl/L in NSW.

Manufacturer	Detergent	Initial Fluorescence (µL/L Tide equivalent)	Reduction in Fluorescence after UV exposure (%)				OB Detection	
			2 min	5 min	10 min	30 min	Hartel Method	Revised Method
Bi-O-Kleen	All Temperature	5.3	0.0	1.6	4.5	13.2	No	No
Church & Dwight	Xtra Lasting Sensations	16.2	8.4	9.6	12.9	25.2	No	Yes
Earth Friendly Products	ECOS Ultra Original Formula	9.2	6.0	8.9	13.0	26.3	No	Yes
Kirkland	Free & Clear	7.3	8.9	13.9	13.4	21.6	No	Yes
	Ultra Free & Clear	7.2	8.8	11.5	13.6	23.1	No	Yes
Proctor & Gamble	Arm & Hammer Clean Burst	47.8	10.2	12.5	15.3	26.1	No	Yes
	Cheer	11.2	42.4	42.4	44.7	48.2	No	Yes
	Cheer Color Guard	6.3	18.0	21.0	24.6	33.1	No	Yes
	Gain	9.5	37.0	38.4	40.5	46.0	No	Yes
	Tide Clean Breeze	17.4	56.1	57.1	57.4	61.9	Yes	Yes
	Tide Clean Breeze 2X Ultra	27.4	64.0	65.2	65.6	68.5	Yes	Yes
	Tide Free	17.1	54.4	55.4	56.2	61.4	Yes	Yes
	Tide Original Scent	16.5	52.0	53.3	54.3	59.4	Yes	Yes
	Tide with Bleach	28.1	37.3	38.5	40.1	46.2	Yes	Yes
	Tide with Bleach Alternative	25.9	37.3	38.4	39.8	45.3	Yes	Yes
Trader Joe's	Concentrated Detergent	13.2	8.2	9.6	16.4	35.8	No	No
Unilever	All Free Clear	15.6	51.7	52.9	53.8	59.9	Yes	Yes
	All Fresh Rain	15.4	51.8	51.7	56.3	59.4	Yes	Yes
	All Small & Mighty	39.7	69.3	70.4	70.1	71.7	Yes	Yes
	Ultra (2X Ultra All)	30.9	68.9	69.2	69.4	71.3	Yes	Yes
	Wisk Ultra	15.9	53.5	54.4	54.8	60.0	Yes	Yes

in the dark, at room temperature. Fluorescence degradation was also relatively low when samples were held in the shade in the morning (Table 4). However, later in the day, samples in the sunlight degraded rapidly after the UV index reached 2; even samples in the shade degraded more than 25% when the UV index reached 7, presumably from reflective radiation.

Discussion

The Hartel *et al*. (2007a) method of using UV exposure to distinguish OB from interfering natural background compounds was found to be valid in the present study, as the method produced no false positives. All OB sources tested had photodecay curves characterized by sharp initial drops in fluorescence, while samples without OB had very little decay. However, the present study also found the method to be insensitive, in that it failed to detect raw sewage and approximately one-half of the detergents tested in natural stream water at 5 µl/L.

Assessment of sensitivity depends on the concentrations tested. For the present study, the rationale for testing detergents at 5 µl/L was that 50 ml of detergent is typically placed into a 100-L wash load, yielding 500 µl/L (with a conservative assumption of no absorption to clothes) in the washing machine. A 100-fold dilution from other plumbing systems with-

Table 3. Photodecay characteristics of sewage and septage samples. All sewage samples were diluted 10-fold in NSW. Septage samples were diluted 20- or 100-fold in DI.

Sources	Initial Fluorescence (µL/L Tide equivalent)	Reduction in Fluorescence after UV Exposure (%)				OB Detection	
		2 min	5 min	10 min	30 min	Hartel Method	Revised Method
Septage[a]	45.0	32.0	44.4	51.8	60.0	Yes	Yes
Septage[b]	10.0	35.0	47.7	54.2	64.0	No	Yes
OCSD Influent	12.9	11.9	15.7	18.6	27.8	No	Yes
OCSD Primary Effluent	10.7	5.8	9.4	12.4	22.4	No	Yes
OCSD Secondary Effluent[c]	9.1	3.3	7.5	10.8	21.2	No	No
OCSD Secondary Effluent[d]	9.0	3.4	6.9	10.9	24.0	No	No
Encina Influent	9.7	6.0	9.1	12.7	21.9	No	UD (2/3)[e]
Encina Primary Effluent	8.0	2.7	6.1	9.9	22.2	No	No
Morehead Influent	10.2	4.8	7.8	12.0	20.9	No	UD (2/3)
Morehead Primary Effluent	8.6	n/a	5.8	8.5	18.9	No	No

[a] Diluted 20-fold in DI

[b] Diluted 100-fold in DI

[c] Following trickling filter treatment

[d] following activated sludge treatment

[e] Undetermined: only 2 out of the 3 replicate samples were positive

in the house and subsequent dilution in streams was assumed. This is consistent with typical 7 - 20 µg/L OB concentration in sewage influent (Kramer *et al*. 1996, Poiger *et al*. 1996, Poiger *et al*. 1998), which translates to approximately 5 - 13 µl/L detergent concentration with the assumption that OBs are added to detergents at an average concentration of approximately 0.15% by weight (Hayashi *et al*. 2002).

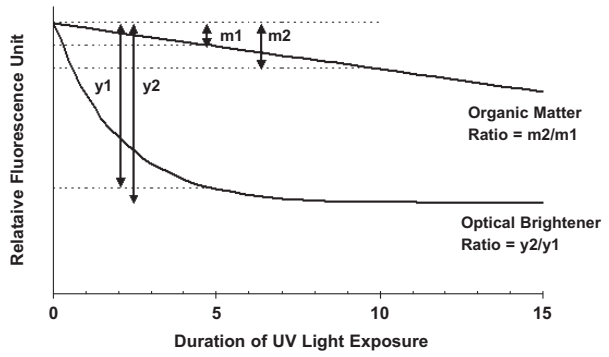

Figure 1. Diagram for demonstrating the usage of ratio to characterize the difference of shapes of the photodecay curves between OBs and NOM. Duration of UV exposure given in minutes.

The present study was not only able to identify a method modification based on the Hartel *et al*. principle of using UV exposure to differentiate background compounds, but also increased sensitivity by using the shape of the photodecay curve in combination with the magnitude of decay, rather than using the magnitude of decay alone. Photodecay curves of OBs were characterized by rapid drops in fluorescence within two to five minutes and subsequent flattening for the remainder of the UV light exposure period, whereas samples without OB had either very little decay or linear-like photodecay curves (Figure 1). Thus, the ratio of reduction after 10 minutes of UV light exposure to reduction after 5 minutes of UV exposure can be used as an additional, more specific means for differentiating OB fluorescence from that of natural organic matter. The present study suggests replacing the Hartel *et al*. step requiring a 15% fluorescence reduction after five minutes of UV exposure with two steps (Figure 2). Step 1: if reduction in fluorescence after 5 minutes is no less than 30%, conclude that the sample contains OBs; if reduction is less than 8% after five minutes, conclude that the sample is negative for OBs. For fluourescence reduction no less than 8% and less than 30% after

Table 4. Signal stability of 5 µl/L mixed detergent in natural environment.

		Duration of Sun Exposure (minutes)									
		5	10	30	60	90	120	150	180	240	300
Morning (8:30 a.m. - 1:30 p.m.)	**UV Index**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**7 - 8**	**7**
	Sun	6	8	24	42	53	64	69	73	80	85
	Shade	1	1	6	12	15	18	23	26	41	43
Around Noon (12:04 p.m. - 4:04 p.m.)	**UV Index**	**7**	**7**	**7**	**8**	**7**	**7**	**6**	**5**	**3**	**-**
	Sun	46	51	65	75	82	85	87	89	91	-
	Shade	40	40	47	52	57	62	65	67	71	-

5 minutes of UV exposure, Step 2 is required. Step 2: if the ratio of reduction after 10 minutes of UV exposure to reduction after 5 minutes of UV exposure is no less than 1.5, conclude that the sample is negative for OBs; otherwise conclude that the sample contains OBs. The second step uses the shape of the curve to segregate those samples for which the photodecay response after the initial five minutes is ambiguous.

The present study also found that the initial fluorescence threshold used in Hartel *et al*. (2007a), which was based on TOC measurement, can be replaced by a fixed lower threshold given the effectiveness of the two-step method for differentiating OB from natural background fluorescence. The lower initial threshold used in the two-step method reduces false negatives caused by the more conservative initial fluorescence threshold used by Hartel *et al*. This modification also relieves the cost of measuring TOC and standardizes the initial threshold across applications.

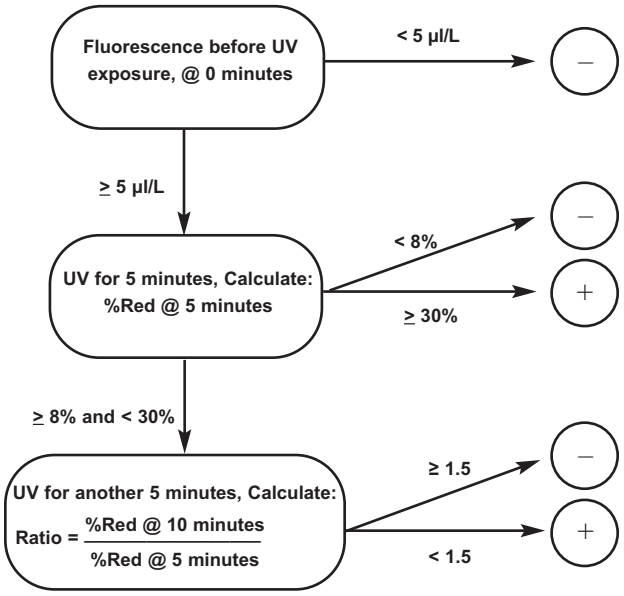

Figure 2. A modified Hartel *et al*. (2007a) method for utilizing the rapid photodecay characteristics of optical brighteners for detection of human waste contamination.

Implementing these method modifications (as outlined in Figure 2) resulted in detection of all but three detergents at 5 µl/L (Table 2). Similarly, the method modification detected septage at 1:100 dilution, which could only be detected at 1:20 dilution using the original method (Table 3). The present study also found that the method modification allowed for detection of sewage influent from all plants at 1:10 dilution, although it was only able to identify sewage effluent from one plant. The method modification produced no false positives among the negative controls.

Improvement primarily resulted from the institution of the ratio step. The Hartel *et al*. threshold of 15% reduction after five minutes of UV exposure is intended to prevent false positives but results in nearly one-half of the positive samples being classified as negative. The ratio approach correctly identified more than 90% of these samples, while maintaining a 100% correct classification for the negative controls. This increased level of certainty also allows for a lower initial threshold, further improving overall classification accuracy.

The improvement in the method was even found to be robust to the type of fluorometer used. Our testing focused on use of the relatively expensive,

laboratory-based 10-AU unit, but we ran parallel testing for a subset of the samples using a less expensive, easy to operate handheld fluorometer (Aquafluor; Turner designs, Sunnyvale, CA) calibrated in the same way as the 10-AU except that the Aquafluor had no sensitivity setting. The present study found no difference in sample classification with respect to OB between the two. This is a potentially big advantage, allowing collection teams to process samples in the field and more effectively pursue a gradient signal.

While this modified approach substantially increased the applicability of the method, it was still less effective for sewage than Hartel *et al*. (2007a) found, which may reflect differences in the sewage tested between our studies. The OCSD influent is about 20% industrial waste, whereas the samples in the Hartel *et al*. study were from rural regions where detergents, perhaps even different ones, may constitute a larger fraction of the sewage. There are also caveats that remain for using the method with septage. While the method was effective with the septage we tested, there were clear differences in response among detergents, with low sensitivity for some environmentally-friendly detergents. This does not present a problem on a community basis where a mix of detergents are used, but could be of concern for assessing inputs from individual homes.

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