Using submersible fluorescence sensors to track the removal of organic matter in decentralized wastewater treatment systems (DEWATS) in real time

N. Mladenov, A. Bigelow, B. Pietruschka, M. Palomo and C. Buckley

ABSTRACT

Decentralized wastewater treatment systems (DEWATS) using anaerobic treatment are increasingly being considered for wastewater treatment with options for non-potable water reuse at the community scale. One challenge for ensuring performance and reliability of DEWATS is the lack of suitable on-site sensors to monitor failure or contamination events. In this study, the aim was to use *in situ* fluorescence sensors to track the performance of a DEWATS, consisting of an anaerobic baffled reactor (ABR) coupled to anaerobic filter (AF) and constructed wetland (CW) treatment processes. A submersible *in situ* fluorometer equipped with tryptophan (TRP) and chromophoric dissolved organic matter (CDOM) sensors was deployed in each chamber of the ABR-AF-CW system, and results showed that TRP fluorescence was preferentially removed over CDOM fluorescence throughout the system. Significant relationships between TRP fluorescence and chemical oxygen demand (COD) also suggested that TRP fluorescence could be used as a surrogate for COD and soluble COD concentrations. Strong agreement between results obtained from the 1D *in situ* fluorometer and those obtained from a 3D benchtop fluorometer lends further support to the use of *in situ* fluorescence sensors to track DEWATS performance.

Key words | ABR, anaerobic, decentralized, fluorescence, wastewater, water reuse

N. Mladenov (corresponding author)
A. Bigelow
Department of Civil, Construction, and Environmental Engineering,
San Diego State University,
5500 Campanile Drive, San Diego, CA 92182,
USA
E-mail: *nmladenov@sdsu.edu*

B. Pietruschka
Bremen Overseas Research and Development Association,
Am Deich 45, 28199 Bremen,
Germany

B. Pietruschka
C. Buckley
School of Engineering, University of KwaZulu-Natal,
Howard College Campus, Durban, 4041,
South Africa

M. Palomo
Department of Civil Engineering,
California State Polytechnic University,
3801 West Temple Avenue,
Pomona,
CA 91768,
USA

INTRODUCTION

Worldwide, population growth has outpaced infrastructure growth in many urban settings, leaving communities without adequate water and sanitation services. There is both the challenge of providing formalized water and wastewater treatment for growing populations and the burden of replacing aging infrastructure. In contrast to large-scale centralized wastewater treatment systems, decentralized wastewater treatment systems (DEWATS) treat hundreds or thousands of litres per day instead of millions and on a much smaller footprint. Therefore, DEWATS now have an important potential role in providing wastewater treatment with options for water reuse at the community scale.

Anaerobic baffled reactor (ABR) technology, originally developed by McCarty at Stanford (McCarty 1981) is the core module of DEWATS that has recently gained attention for the treatment of domestic wastewater (Foxon *et al.* 2004; Hahn & Figueroa 2015). Anaerobic wastewater treatment, such as secondary treatment using ABR systems, has low maintenance and energy requirements and generates a fraction of the residuals compared to aerobic systems, resulting in reduced sludge disposal. ABR systems have been installed by the Bremen Overseas Research and Development Association (BORDA) for use at the community level in Durban, South Africa and at the individual household level in Maseru, Lesotho (with a mean annual temperature of 15 °C) to treat wastewater while generating biogas, and producing water used to irrigate property (gardens and lawns). The household ABRs in Lesotho have been successfully operated even at psychrophilic temperatures. Similarly Hahn & Figueroa (2015) observed efficient chemical

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oxygen demand (COD) removal for a pilot-scale ABR system operating at psychrophilic temperatures in Colorado, USA. Also, due to their low energy or potentially energy-positive nature, anaerobic treatment processes can potentially alleviate the current high energy demands from the centralized, publicly owned treatment works (POTW) sector.

ABR performance is based on the up-flow anaerobic sludge blanket (UASB) design in which wastewater flows longitudinally across chambers separated by vertical baffles (Figure 1). After passing downward into the baffle, wastewater flows upward through a fluidized anaerobic sludge region in each chamber. The ABR has been described as a series of connected UASBs (Barber & Stuckey 1999) or serial septic tanks (Sasse 1998). Septic tanks typically remove 30–50% of the 5-d biochemical oxygen demand (BOD_5) and 60–80% total suspended solids (TSS) (Lowe 2007; US EPA 2002). Similarly, ABRs have been shown to have removal rates on the order of 50% for both BOD_5 and COD and ~70% for TSS removal (Singh *et al.* 2009). Coupling of the ABR system with post-treatment polishing steps such as the anaerobic filter (AF) for removal of soluble COD, and constructed wetlands (CW) for nutrient removal (ammonia) and further effluent polishing is a standard approach in several developing countries, which can provide much higher removals. For example an ABR coupled to horizontal flow and vertical flow CW was able to reach 77% removal for COD and 91% removal for TSS (Singh *et al.* 2009). Biofilms grow on the rocks within AF systems and can result in sorption of soluble compounds to the biofilm and degradation of compounds by microbial communities in the biofilm.

For ABR-based systems, such as DEWATS treating domestic wastewater at the community scale, both wastewater generation and application of the treated effluent or reclaimed water will occur in closer proximity to the DEWATS than in the case of centralized POTW systems. Therefore, for water reuse or reclamation applications, holding times may be low or non-existent, offering little time to monitor system failure or contamination events. One solution to address this issue is the use of real-time sensors to ensure treatment systems meet local performance and

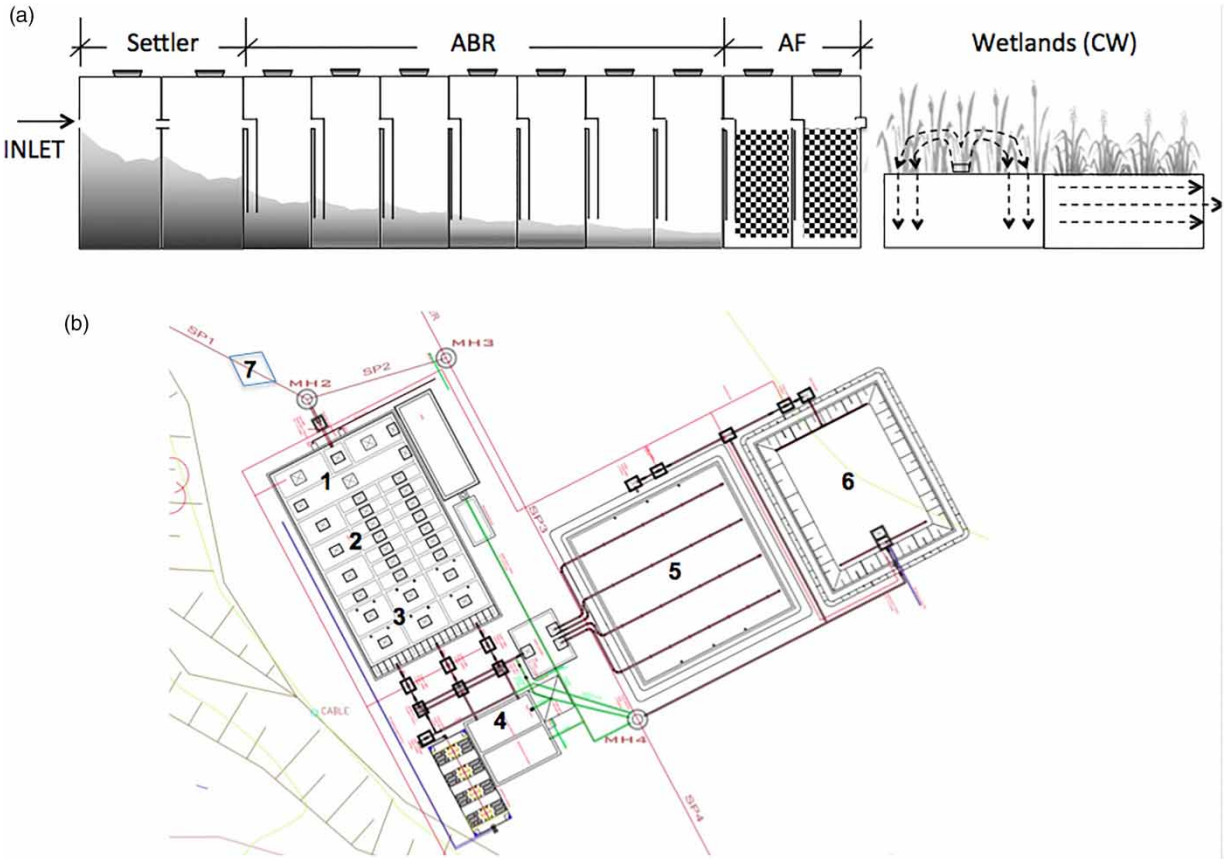

Figure 1 | Profile view (a) and top view (b) of ABR-AF-CW system. The dominant processes expected to be underway in this treatment system are shown below each treatment type. In the top view, numbering is as follows: (1) two settling chambers, (2) three parallel trains of ABR chambers, (3) three parallel trains of two AF chambers, (4) membrane sump, (5) VGF, (6) HGF and (7) stormwater bypass.

effluent standards. Indeed, in terms of evaluating DEWATS performance, one of the main challenges BORDA is facing is the current lack of suitable on-site sensors (Schmidt and Pietruschka, personal communication). Of all the real-time sensors available, fluorescence holds promise due to its ability to discriminate several classes of organic compounds and provide *in situ* and instantaneous measurement. Fluorescence is a measure of the intensity of energy emitted (in the UV and visible range) by excited molecules that absorbed light energy (excitation). Fluorescence has been used for surface water quality monitoring since the 1970s (e.g., for oil spill tracking (John & Soutar 1976)). Recently, fluorescence has also found monitoring applications in wastewater treatment systems (Hudson *et al.* 2007; Carstea *et al.* 2016; Sgroi *et al.* 2017a) and in tracking of wastewater inputs to aquatic ecosystems (Goldman *et al.* 2012; Hur & Cho 2012; Sgroi *et al.* 2017b) and pathogenic bacterial loads to rivers (Baker *et al.* 2015). *In situ* or portable fluorescence sensors have the potential to become a valuable tool for monitoring reliability and performance of DEWATS for water reuse. To our knowledge, fluorescence sensors have not yet been applied in such settings.

Organic compounds in natural and engineered systems have been examined extensively with 3D benchtop fluorometers, which produce fluorescence intensity measurements at hundreds of excitation–emission wavelength pairs, visualized as an excitation–emission matrix (EEM). Benchtop instruments are more sensitive than *in situ* sensors and have the advantage of tracking multiple fluorophores at once, including peaks associated with tryptophan-like (TRP) fluorescence and chromophoric dissolved organic matter (CDOM) fluorescence associated with humic and fulvic acids. However, spectra acquired with benchtop instruments require calibrations and instrument-specific corrections and the instrument itself is expensive and has poor portability compared to *in situ* sensors. To test the extent and limits of portable 1D fluorescence sensors for contaminant tracking, more work is needed to 'ground-truth' the 1D measurements with more sensitive 3D fluorescence acquisition.

The purpose of this study was to use portable and benchtop fluorescence instruments to assess the efficiency of parallel ABR systems in removing organic matter (OM). We deployed an *in situ* 1D submersible fluorometer to track OM degradation in a fully anaerobic DEWATS (Figure 1). Results of the *in situ* instrument and multiple water quality characteristics, including COD and dissolved organic carbon (DOC) concentrations, were compared to results of the 3D benchtop fluorometer to evaluate agreement among both instruments for tracking ABR performance.

METHODS

Sample collection

Samples were collected from the DEWATS treating wastewater from 84 homes in a low-to-middle income community near Durban, South Africa. The system includes a two-chamber settler (no. 1, Figure 1) and 1 four-chamber and 2 seven-chamber ABRs operating in parallel (no. 2, Figure 1). All ABRs are coupled to a two-chamber AF (no. 3, Figure 1). Effluent from the first seven-chamber ABR-AF is discharged into CW consisting of a planted vertical gravel filter (VGF) and horizontal gravel filter (HGF) (no. 5 and no. 6, Figure 1; Supplementary Figure S1, available with the online version of this paper). Effluent from Trains 2 and 3 is fed back into the municipal system. The volumes of each train, design flowrates, and average flowrates for July 2015 are shown in Table 1. Average hydraulic residence times in each 3.06 m^3 ABR chamber were typically 3–4 h.

Grab samples were collected for OM analysis from ABR chambers 1 (ABR inlet) through 7 (ABR outlet), AF chambers 1 and 2, and HGF effluent. No influent samples could be collected from the influent stream prior to flow splitting into three trains. After collection, samples were filtered through a glass fiber filter (GFF)-style filter (Millipore) with 0.7 μm nominal pore size and transported chilled to San Diego State University for OM analysis and fluorescence spectral acquisition using the benchtop fluorometer.

Grab samples were also collected from five ABR-AF-CW systems treating wastewater from individual households in Maseru, Lesotho. Samples were collected from the inlet of the ABR, inlet of the CW, and outlet of the CW. Samples

Table 1 | DEWATS volumes and hydraulic loadings

	DEWATS volumes (m^3)				Flowrates (m^3/d)	
	ABR chamber[a]	ABR train	AF	Total	Design	07/2015 Average
Settler	–	–	–	37.4		
Train 1	3.06	21.4	6.13	33.7	13.9	5.09
Train 2	3.06	21.4	6.13	33.7	13.9	1.58
Train 3	3.06 (6.13)	21.4	6.13	33.7	13.9	10.0
Total		64.3	18.4	138	41.6	16.7

[a]Chambers 1–3 in Train 3 are double sized with volume of each shown in parentheses. The fourth chamber of Train 3 has the same volume as each chamber in Trains 1 and 2.

were transported immediately to the laboratory at the University of KwaZulu-Natal, filtered through a GFF-style filter (Millipore) with 0.7 μm nominal pore size, and transported chilled to San Diego State University for OM analysis and fluorescence spectral acquisition using the benchtop fluorometer. The *in situ* fluorometer was not deployed in the Lesotho ABRs.

Organic matter analyses and monitoring

Grab samples collected from Trains 1 and 3 were analyzed at the on-site laboratory for COD and soluble COD (sCOD) concentrations. No samples were collected from Train 2 for COD analysis. COD samples, which include both particulate and sCOD, were diluted (1:5) with deionized water prior to analysis using a closed reflux, titrimetric method. Samples were digested with potassium dichromate and sulfuric acid in an Ethos One high performance microwave digestion system. Soluble COD samples without dilution were centrifuged, and the supernatant was digested similarly to the COD. Both COD and sCOD were then titrated with ferrous ammonium sulfate.

DOC was measured as non-purgeable organic carbon using a high temperature oxidation method on a Shimadzu TOC-L_{CPN} total organic carbon analyzer. Detection limits for DOC concentration analysis are 0.050 mg C/L.

In situ fluorescence measurements were recorded using a 1D portable sensor, the C3^{TM} submersible fluorometer (Turner Designs), equipped to detect humic substance fluorescence (referred to using manufacturer terminology as 'CDOM' fluorescence) at excitation/emission (ex/em) wavelengths centered at 325/470 nm and TRP fluorescence at ex/em wavelengths centered at 285/350 nm. *In situ* data acquisition consisted of submerging the C3 to 1 m depth in each chamber of the ABR, AF, and HGF outlet (Train 1 only). The C3 was agitated slightly to dispel any air bubbles and then held still to record fluorescence data. Triplicate readings were taken, repeating the measurement three times at each sampling location.

Temperature corrections were not applied to the fluorescence data due to the narrow temperature range (average temperatures of 22.5 °C to 23.2 °C across all trains). This narrow range does not have a measureable influence on TRP or CDOM fluorescence intensities (Wasswa and Mladenov submitted). Calibration curves were developed for both TRP and CDOM concentrations using commercially available tryptophan (Sigma Aldrich, L-Tryptophan, reagent grade) and Suwannee River (International Humic Substances Society) CDOM. Uncalibrated and calibrated results are reported in both relative fluorescence units (RFU) and tryptophan standard equivalents (TSE), respectively.

Benchtop fluorescence data included single point intensities measured at the same ex/em wavelengths for TRP (285/350 nm) and CDOM (325/470 nm) using a Horiba Aqualog spectrofluorometer. Three dimensional excitation–emission matrix spectra (EEMs) were acquired at excitation wavelengths ranging from 240 to 450 at 3 nm increments and emission wavelengths ranging from 300 to 600 nm at 4.5 nm increments. All samples were corrected for the inner filter effect and first and second order Raleigh scattering, Raman normalized, and blank-subtracted. EEM post-processing was performed using Matlab. In addition to fluorescence intensities of the TRP and CDOM (also referred to as humic-like Peak C) wavelength pairs, the tyrosine-like (TYR) fluorescence wavelength at 275/310 nm was also visible in EEMs of ABR, AF, and CW samples. These wavelength pairs have been widely observed and reported in the environmental fluorescence literature (e.g., Coble 1996; Fellman *et al.* 2010). The humification index (HIX), which provides information about the degree of humification of dissolved organic matter (DOM), was calculated as the ratio of peak area under the emission spectra at 435–480 nm to peak area at 300–345 nm obtained at an excitation wavelength of 254 nm (Zsolnay *et al.* 1999).

RESULTS AND DISCUSSION

Removal of organic matter along the ABR-AF-CW system

Flowrates in the ABR-AF-CW system had daily peaks at ~11:00, and many dates exhibited no flow during early morning hours (Figure 2). There is similar variability in the organic loading in DEWATS treating domestic wastewater due to household patterns of wastewater generation. Such variability is evident when comparing annual means and standard

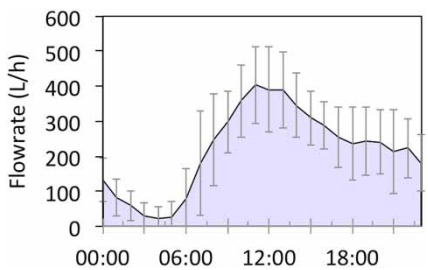

Figure 2 | Average hourly flowrates ($n = 37$ measurements) recorded at the outlet of Train 1 from 25 June to 31 July 2015. Error bars represent one standard deviation from the mean.

deviations of COD concentrations measured at the ABR inlet with those measured in the AF outlet from January to December 2015 (Figure 3). Over the course of 1 year, ABR inlet COD concentrations ranged from 378 mg/L to almost 2,000 mg/L, whereas CW outlet concentrations were in a narrower range, from 16 mg/L to 159 mg/L (Figure 3). Even during the course of the study, COD concentrations measured in the ABR inlet for Trains 1 and 3 were highly variable, ranging from 390 to 800 mg/L with mean inlet COD concentrations at ~500 mg/L (Figure 4(a)).

The COD concentrations we measured for domestic wastewater from the low-to-middle income community in this suburb of Durban were in the range of other studies using ABR technology to treat domestic wastewater. For comparison, COD in the influent of a carrier ABR treating domestic sewage from university dormitories in Zhejiang Province, China has been reported at ~300 mg/L (Huajun *et al.* 2008), and COD in the influent of a pilot ABR system treating municipal wastewater in Colorado, USA was on the order of 700 mg/L (Hahn & Figueroa 2015).

Due to the diurnal variability in the concentration of organics entering the system, and the tanks-in-series nature of the ABR, there can be substantial variability in terms of removal efficiency in the ABR-AF-CW system. Indeed, at some times of day, COD concentrations in the inlet to ABR 1 could be lower than those measured in subsequent chambers due to this variability in organic loading, and this scenario poses a challenge to calculating removal efficiencies in the system. The approach we used was to determine the difference between average ABR outlet concentrations and average inlet concentrations on multiple dates. Evaluating removal efficiencies in this way allowed us to avoid the diurnal variability.

Student t-tests indicate that COD removal rates for the seven-chamber Train 1 ABR (at 48%) were significantly higher ($p < 0.01$) than for the four-chamber Train 3 ABR (at only 18%; Table 2). Although the higher volume of wastewater flowing into Train 3 results in a higher organic loading

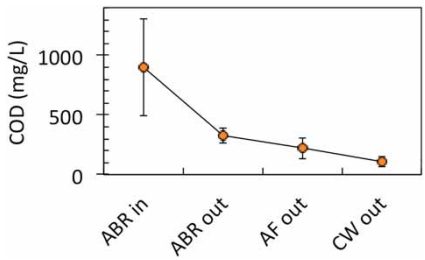

Figure 3 | Mean annual COD concentrations ($n = 24$ measurements) in ABR inlet and outlet, AF outlet, and CW outlet from 1 Jan to 31 Dec 2015. Error bars represent one standard deviation from the mean.

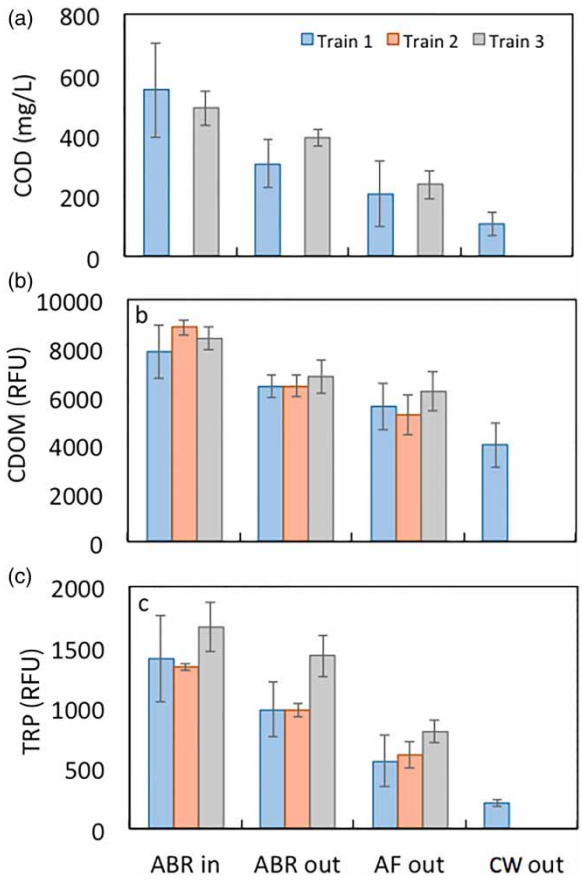

Figure 4 | Change in (a) COD concentration, (b) CDOM fluorescence, and (c) TRP fluorescence from inlet of the ABR to outlet of the ABR, AF, and CW for Trains 1, 2, and 3. Trains 2 and 3 did not flow into the CW, and, as a result, data are not available for those trains.

rate in this train, the additional degradation that occurs in Trains 1 and 2 is likely a function of the additional chambers. The shorter horizontal path in the seven-chamber ABR results in reduced bacteria washout (compared to fewer, larger chambers) and, in turn, longer solids retention time and more efficient OM degradation. Foxon *et al.* (2004) used an eight-chamber pilot ABR system for the treatment of municipal wastewater with similar influent COD concentrations (at ~560 mg/L) and found COD removal (at 58%) that was also substantially higher than what we observed for the four-chamber Train 3 ABR.

Additional treatment by rock media in the AF follows ABR treatment (Figure 1). The AF process provided high COD removal, especially for Train 3 (at 31%). The higher COD removal by the AF in Train 3 made up for the low removal in the ABR and raised the total COD removal to 49% for Train 3 compared to 59% for Train 1 (Table 2).

DOC concentrations, measured for only one sampling date, were in the range 35–52 mg/L in the ABR inlet,

Table 2 | Average removal efficiencies of COD, sCOD, DOC, and *in situ* CDOM and TRP fluorescence in the ABR, AF, and CW treatment steps[a]

			% Removal				
Train	**Parameter**	**n**	**ABR**	**Anaerobic filter**	**Total**	**Constructed wetland**	**Total**
1	COD	6	48 ± 9.7%	12 ± 10%	59%	25 ± 21%	84%
	sCOD	7	32 ± 4.2%	19 ± 8.3%	51%	16 ± 2.8%	67%
	DOC	1	30%	12%	42%	16%	58%
	CDOM	7	21 ± 6.8%	15 ± 11%	36%	30 ± 24%	66%
	TRP	7	29 ± 8.6%	28 ± 10%	57%	30 ± 16%	88%
2	COD	0	NC	NC	NC	–	–
	sCOD	0	NC	NC	NC	–	–
	DOC	1	17.1%	29.4%	47%	–	–
	CDOM	2	27 ± 2.7%	14 ± 4.8%	41%	–	–
	TRP	2	27 ± 2.6%	27 ± 12%	54%	–	–
3	COD	6	18 ± 12%	31 ± 11%	49%	–	–
	sCOD	3	21 ± 11%	33 ± 6.9%	54%	–	–
	DOC	2	3.5 ± 7.4%	34 ± 9.8%	37%	–	–
	CDOM	8	19 ± 9.8%	13 ± 8.1%	32%	–	–
	TRP	8	14 ± 1.1%	37 ± 2.4%	51%	–	–

[a]± standard deviations. Dash indicates that no data existed for Trains 2 and 3 because only Train 1 was followed by CW treatment. *n* = number of samples; NC = samples not collected for COD or sCOD in Train 2.

24–43 mg/L in the ABR outlet, and 20–28 mg/L in the AF outlet of the three treatment trains (Table 2). DOC concentration at the outlet of the CW was 15 mg/L, which represents a 58% decrease in DOC along the ABR-AF-CW treatment system (Table 2).

CDOM and TRP fluorescence intensities of the influent were also variable, consistent with the wide range in COD concentrations. CDOM fluorescence intensities ranged from ~6,500 to 9,000 RFU, with a mean of ~8,000 RFU (Figure 4(b)). TRP fluorescence intensities ranged from 900 to 1,900 RFU (2.5 to 3.5 mg/L as TSE), with a mean of ~1,500 RFU (Figure 4(c)). Previous research in waterways influenced by wastewater discharge identified TRP fluorescence as a marker of microbial influences, and reported TRP intensities as high as 0.10–0.20 mg/L as TSE at several sites with correspondingly high *Escherichia coli* numbers (Baker *et al.* 2015).

Consistent with COD results, both CDOM fluorescence and TRP fluorescence removals were higher in the seven-chamber ABRs (Trains 1 and 2) than in the four-chamber ABR (Train 3) (Table 2). The TRP fluorescence decrease along the ABR (which was more significant than the decrease in humic (CDOM) fluorescence) supports our findings of improved COD removal efficiency in the seven-chamber ABR. This preferential decrease in TRP fluorescence suggests that additional chambers result in improved removal of biodegradable OM in the system.

Treatment underway in the AF chambers was also very effective at removing OM with TRP fluorescence, especially for Train 3. It is known that biological solids trapped in interstitial spaces of rocks, stones, or other media in the AF provide a seed for biodegradation of organic compounds in wastewater (Young & McCarty 1969). Although TRP removal in Train 3 had been low in the ABR, the TRP removal by the AF was high in Train 3 (Table 2). By contrast, AF treatment resulted in only 13–15% removal (on average) of CDOM fluorescence, compared to 27–37% removal (on average) for TRP fluorescence. Even when considering the full ABR-AF-CW system, removal of CDOM fluorescence (at 66%) was much lower than that of TRP fluorescence at 80%. The spatial variability of TRP fluorescence was similar to that of COD throughout the system, which was also reduced by about 80% in the outflow of the full ABR-AF-CW system.

The greatest removal of CDOM fluorescence was measured in the CW cells (Figure 5). The movement of water through the CW promotes subsurface contact of water with wetland soils, whereby sorption and redox reactions control the degradation of organic compounds (Vymazal 2005). The substantial decrease in CDOM fluorescence in the CW (at ~30% removal; Table 2) suggests that compounds responsible for both TRP and CDOM fluorescence were removed in the wetlands. The latter tend to be larger molecular structures, such as fulvic acids, that are more recalcitrant to biodegradation, and were therefore less amenable to biodegradation in earlier ABR and AF stages. Another important change in the ABR-AF-CW

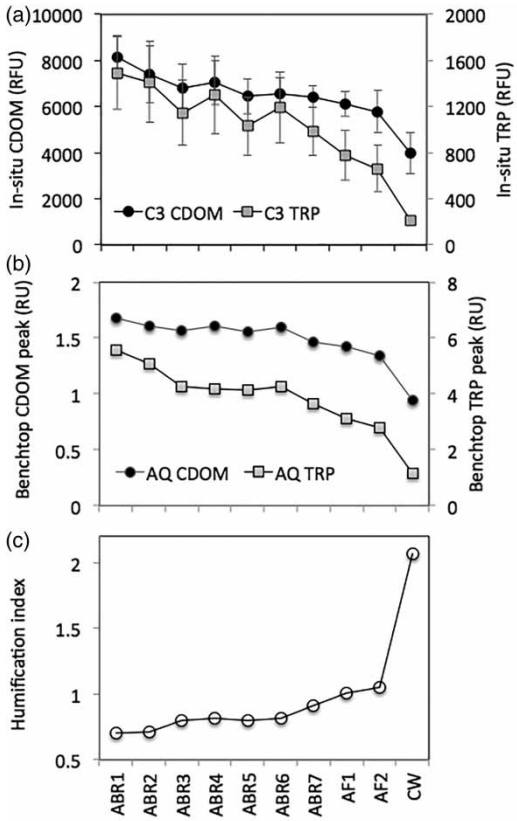

Figure 5 | Change in (a) mean fluorescence intensities of CDOM and TRP peaks acquired on multiple dates with an *in situ* (C3) fluorometer, (b) CDOM and TRP fluorescence for samples collected on one date with an Aqualog (AQ) benchtop fluorometer, and (c) HIX of spectra acquired using the benchtop fluorometer.

system was the large increase in humic OM, represented by the increase in the HIX (Figure 5(c)). The HIX increases as labile and lower molecular weight compounds are removed or degraded, or high molecular weight humic compounds are produced or released from soils. In our study, the HIX increased from ~0.7 at the ABR inlet to >2.0 at CW outlet (Figure 5(c)). Similarly, in their study of the characteristics of fluorescent OM in a biological pond and CW system for domestic wastewater in China, Yao *et al.* (2016) also found an increase in the fluorescent peaks associated with high molecular weight humic substances and concluded that wetland treatment induced humification and chemical stability of the DOM.

Comparison of *in situ* and benchtop fluorometers for OM tracking

TRP fluorescence acquired using the *in situ* fluorometer was compared with TRP fluorescence measured at the 285/350 nm ex/em wavelength pair using the benchtop fluorometer. Similarly, *in situ* CDOM fluorescence was compared with Peak C fluorescence measured at the 325/470 nm ex/em wavelength pair. We found that TRP and CDOM fluorescence profiles along the ABR-AF-CW system showed close agreement for both *in situ* and benchtop instruments (Figure 5(a) and 5(b)). Consistent with the findings using the *in situ* instrument, results from the benchtop instrument indicated that ~80% of the TRP fluorescence and ~45% of the CDOM fluorescence was removed between the inlet of the ABR and the outlet of the CW (Figure 5(b)). The reduction in the TRP fluorescence peak is illustrated in EEMs of representative samples collected from the ABR inlet and outlets of the ABR, AF, and CW. Overall there is a reduction in all fluorescent peaks, but the greatest decrease is seen in the TRP and TYR peaks, which decreased from ~18 RFU to ~5 RFU and ~3 RFU, respectively (Figure 6). Benchtop fluorescence results therefore also support that there is preferential removal of labile (or biodegradable) tryptophan-like and tyrosine-like moieties in the ABR-AF-CW system. This finding is consistent with greater removal of protein-like fluorescence observed in a conventional wastewater treatment plant in a study by Sgroi *et al.* (2017a). Using 3D EEMs in combination with compound-specific analysis of trace organic compounds, the authors found higher removal of protein-like

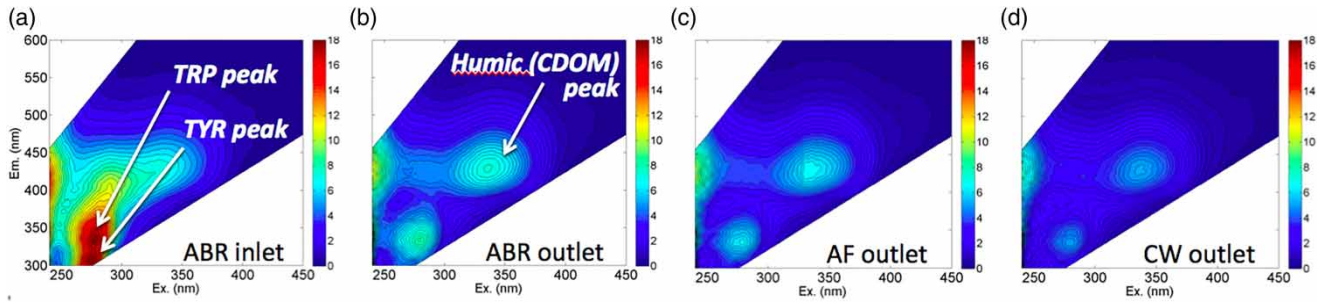

Figure 6 | EEMs of representative water samples collected from (a) ABR inlet, (b) ABR outlet, and (c) AF outlet on 30 June 2015 show preferential reduction in the tryptophan-like fluorescence peak (TRP peak) along the ABR-AF-CW treatment train.

components (on the order of 63–82%) compared to humic-like components (which decreased by only 28–42%) during sewage treatment.

In addition, in our study the much lower overall fluorescence intensities (e.g., four-fold lower TRP and TYR peak intensities) in the EEM of the CW effluent (Figure 6(d)) compared to the EEM of the ABR inlet mean that system failures, which may produce intense fluorescence peaks, are likely to be visible against the typical background fluorescence of the CW effluent. Therefore, 3D fluorescence EEMs may additionally be useful for tracking process upsets in the system.

Relationships between fluorescence and organic matter concentrations

To make use of fluorescence data for tracking OM removal in DEWATS, one important question is 'what quantitative measures of OM do CDOM and TRP fluorescence represent?' CDOM fluorescence, also referred to as Peak C fluorescence in the 3D EEM spectrum (Coble 1996), is known to reflect contributions from lignaceous OM, such as fulvic acids (Fellman *et al.* 2010), and microbially reprocessed OM (Carstea *et al.* 2016). Our study did not quantify fulvic acid content or other measures of more recalcitrant DOM; however, the lower removal of CDOM fluorescence throughout the ABR compared to TRP fluorescence, indicates that compounds fluorescing in the humic-like CDOM region are less labile than those comprising the TRP region.

By contrast, TRP fluorescence is known to represent OM that is predominantly microbial in nature and readily available for degradation. The TRP peak in the EEM (at ex/em ~275/340 nm) has been positively correlated with wastewater and biodegradation parameters such as bacterial respiration (Cammack *et al.* 2004), the presence of *Escherichia coli* (Baker 2001; Baker *et al.* 2015), biodegradable DOC (Fellman *et al.* 2008), BOD (Hudson *et al.* 2008; Hur and Cho 2012), and COD (Hur and Cho 2012). Therefore, its high removal in the ABR chambers, compared to low humic-like fluorescence removal, reflects preferential degradation of labile OM.

Using single point fluorescence intensities retrieved from both *in situ* and benchtop fluorometers in 10 different ABR systems sampled in South Africa and Lesotho, we found significant relationships between COD concentration and both CDOM and TRP fluorescence (Figure 7). However, fluorescence measured in the TRP region correlated more significantly with COD and sCOD concentrations than fluorescence measured in the CDOM region (Figure 7). In

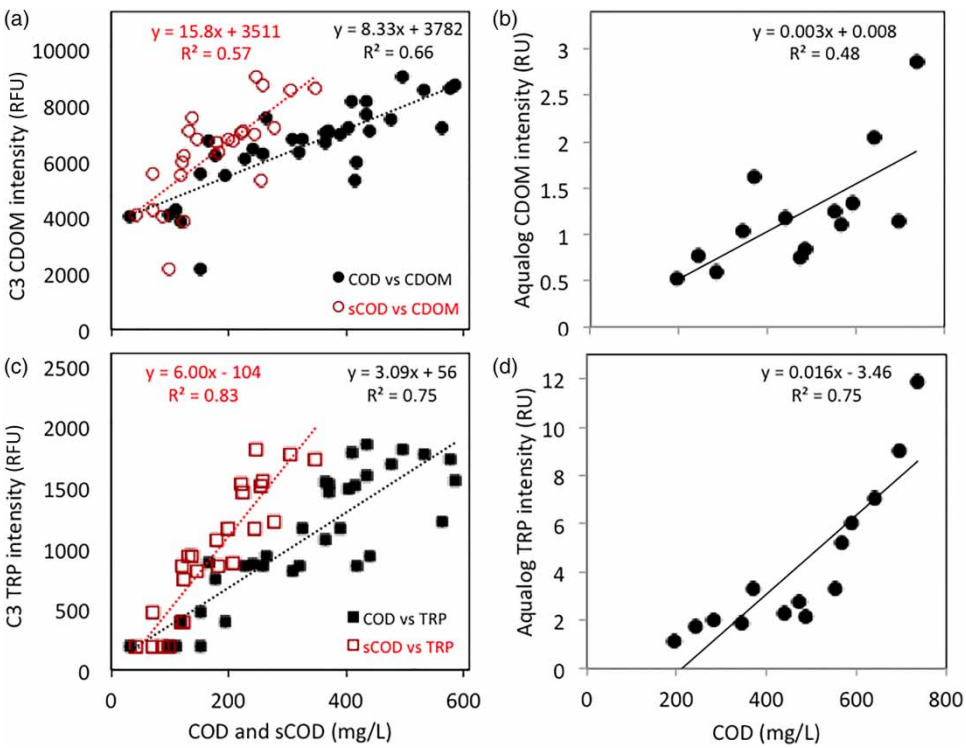

Figure 7 | Relationships between COD and CDOM (top) and TRP (bottom) fluorescence intensities measured in ABR systems using an *in situ* C3 fluorometer ((a) and (c)) and a benchtop Aqualog fluorometer ((b) and (d)). All relationships are significant at the $p < 0.01$ level.

addition, the relationship between TRP fluorescence and COD was significant (R^2 of ~0.75) and similar using both types of fluorometers (Figure 7(c) and 7(d)). This close agreement between the more sensitive benchtop instrument and the portable *in situ* instrument validated the *in situ* data and was an encouraging result, given that the *in situ* instrument is subject to variable environmental factors, such as high and variable turbidity and changes in temperature. Moreover the similar COD–fluorescence relationships for both instruments indicate that the *in situ* fluorescence sensor captures the same patterns in OM quality as the 'gold standard' benchtop fluorometer. These results support the deployment of *in situ* sensors to track the efficiency of OM removal in different DEWATS or within different compartments of the DEWATS treatment train.

CONCLUSIONS

Fluorescence spectroscopy was used to track COD removal in an ABR-AF-CW system. Both CDOM and TRP fluorescence decreased along the ABR-AF-CW system. TRP fluorescent compounds were removed to a greater extent in the ABR and AF chambers, whereas the greatest removal of CDOM fluorescence occurred in the wetland cells. Fluorescence measured using the *in situ* TRP sensor provided a better approximation for COD concentrations than fluorescence measured using the CDOM sensor. Indeed, TRP sensor intensities were significantly correlated with both COD and sCOD, but correlations with sCOD were much higher and more significant. Similar patterns were obtained with a 3D benchtop fluorometer (i.e., highly significant relationships between TRP fluorescence and COD concentration compared to less significant relationships between humic-like fluorescence and COD concentrations). These similarities between results acquired with both *in situ* and benchtop instruments lend further support to the use of *in situ* fluorescence sensors for OM tracking in DEWATS.

In situ TRP fluorescence was also able to discern significant differences in treatment between parallel Trains 1 and 3, which were consistent with the higher COD, sCOD, and DOC removal efficiencies observed in Train 1 than in Train 3. The sensitivity to changes in OM quality among parallel trains further supports that biodegradable soluble constituents along the ABR system can be effectively tracked using the TRP fluorescence sensor.

Overall, our results have immediate relevance for development agencies, such as BORDA, looking to identify suitable on-site sensors to evaluate DEWATS performance in real time. The cost of the submersible fluorometer used in this study, which comes equipped with three sensors and a mechanical wiper to prevent sensor fouling, is in the range of US$10,000 to US$13,000. Data logging equipment or computers are needed for recording fluorescence data, and operators should be trained to use the fluorometer software. At locations with high temperature variability, temperature effects will greatly influence fluorescence data, especially at low temperatures for TRP fluorescence (Wasswa and Mladenov submitted).

By contrast, COD digestion vials or other test kits are much less expensive. However, COD analyses also require a digestion block and spectrophotometer, which may increase the cost by several thousand dollars. In addition, samples must be refrigerated if not analyzed right away, and analyses require preparation by trained personnel. Most COD supplies, including digestion vials and test kits, utilize toxic chemicals, such as dichromate, sulfuric acid, and mercury sulfate, which must be handled in a fume hood by trained laboratory personnel and disposed of as hazardous waste. Therefore the costs, preparation time, sample storage, safety, and training requirements of COD methods must also be taken into consideration.

Coupling *in situ* fluorescence monitoring of ABR influent and effluent with less frequent COD or DOC concentration measurements may represent a reliable solution for tracking ABR efficiency and troubleshooting DEWATS, especially in settings where access to laboratory analytical facilities is limited. The costs of the portable fluorometer would be reduced in circuit-rider scenarios in which one technician is employed to perform testing, troubleshooting, and maintenance on multiple DEWATS.

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