Online fluorescence monitoring of effluent organic matter in wastewater treatment plants

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Wastewater treatment is an energy-intensive operation. Energy consumption is forecast to increase by 60% in the forthcoming decade due to tightened legislation surrounding the discharge of final effluent to watercourses. Treatment plants rely on the time-consuming and unreliable biochemical oxygen demand to assess the quality of final effluent, leading to process inefficiencies. Here, we show that fluorescence spectroscopy is a robust technique for real-time monitoring of changes in effluent quality. We installed three portable fluorimeters for 1 month at the final effluent discharge point of a large municipal wastewater treatment plant. We show that organic matter composition of the wastewater varies diurnally depending on the flow rate and antecedent rainfall. High fluorescence intensity and ammonia are attributed to sewage sludge liquor, which is regularly discharged to the treatment plant. Moreover, elevated fluorescence intensities were recorded as a result of process failure following a power outage. Our study shows that on-line fluorescence analysis is capable of

detecting both minor changes in effluent quality and issues with treatment process performance.

Keywords: real-time monitoring, fluorescence spectroscopy, wastewater treatment plant, organic matter

Introduction

The most significant energy usage in wastewater treatment plants (WwTPs) arises from the vigorous aeration of settled sewage in the activated sludge process (ASP, an aerobic system involving entrainment of air for microbial degradation of organic matter - OM). This process contributes to over 55% of the energy budget associated with wastewater treatment (Environmental Knowledge Transfer Network 2008). Due to the diurnal variations in wastewater flow and load, and lack of rapid and reliable effluent monitoring (Bourgeois et al. 2001; Jouanneau et al. 2014), treatment plants often over-aerate the settled sewage to be certain of achieving regulatory compliance, leading to excessive energy consumption and unnecessary operating costs.

In the past two decades, several studies have demonstrated, through off-line monitoring experiments, the potential of fluorescence spectroscopy for treatment process control (Ahmad and Reynolds 1995; Bridgeman et al. 2013; Cohen et al. 2014; Murphy et al. 2011; Ou et al. 2014; Singh et al. 2012, 2015; Tartakovsky et al. 1996). The technique offers practical advantages, such as: fast measurements, cost-effectiveness, lack of need for reagents, and high sensitivity (Coble et al. 1990; Yang et al. 2015). However, no on-line fluorescence monitoring studies have been performed at WwTPs. To date, Galinha et al. (2011) have undertaken the only real-time monitoring study of wastewater on a pilot scale membrane bioreactor system to predict performance parameters. They found that fluorescence was able to describe influent and effluent chemical oxygen demand (COD), but could not predict other performance parameters. Singh et al. (2015) obtained promising results from an online

monitoring study on two water recycling sites. Using a single-wavelength fluorescence sensor, they were able to prove the robustness of the technique in detecting reverse osmosis membrane fouling and integrity. Moreover, Singh et al. (2015) showed that the sensor was sufficiently sensitive to identify underperformance issues. Real-time monitoring of treated wastewater at WwTPs has been hampered by numerous factors that can interfere with the fluorescence signal: fouling, pH, inner filter effects, temperature and metal ion presence (Henderson et al. 2009; Reynolds 2002). To counteract these issues, regular, time consuming cleaning of contact surfaces or subsequent data corrections are recommended.

Here, we report the first *in-situ* and on-line monitoring of treated wastewater, using three fluorescence portable devices, to test the robustness of the technique and the hypothesis that we can obtain valuable results from a 1-month monitoring experiment without major device cleaning or subsequent data correction. In addition, a laboratory scale activated sludge system was constructed to establish, before the in-situ experiment, the relationship between fluorescence and BOD.

Methodology

The real-time experiment was undertaken for 29 days, from the 10th of August until the 7th of September 2015, at a WwTP located in the West Midlands, UK. The treatment plant serves a region of 450,000 population equivalent and collects on average 120 ML/day of wastewater from various types of sources: household, surface runoff, industrial (soluble oil, chemical laboratory waste, engine cleaning, painting wastes, laundering, meat processing, slaughterhouse, print waste etc.). In addition, the WwTP receives activated sludge mixed liquor from a nearby sewage sludge facility at periodic intervals. During the experiment, liquor was pumped, before noon, on the: 13th, 15th, 21st and 24th of August 2015.

The treatment process train consists of coarse and fine screens at the inlet, six primary sedimentation tanks, 3 activated sludge reactors and 12 final settlement tanks. The primary

treatment step removes solids as well as oil and grease, after which, the remaining wastewater is delivered to the ASP, comprising three basic components: 1) a reactor in which microorganisms are kept in suspension, aerated, and in contact with the wastewater they are treating; 2) liquid-solid separation; and 3) a sludge recycling system for returning activated sludge back to the beginning of the process.

Real-time monitoring

Laboratory scale ASP experiment

Before the in-situ measurements were undertaken, a laboratory scale ASP was constructed to check the feasibility of the method and the relationship with BOD. Settled sewage and returned activated sludge (RAS) were collected twice a week from the WwTP and stored at 4^0 C prior to use. The setup consisted of a feed primary tank (30 L volume), aeration tank (10 L volume) and final settling tank (4 L volume) (Fig. S1). The settled sewage was pumped into the aeration tank at a rate of 11 mL/min. Two aquarium air stones were inserted in the aeration tank to replicate the aeration process and two stirrers ensured a greater degree of mixing. A stirrer was inserted in the final settling tank to ensure settlement of the sludge flocs. The settled sludge was returned to the aeration tank via a peristaltic pump at a rate of 11 mL/min. An average mixed liquor suspended solids (MLSS) concentration of 3,300 mg/l was maintained in the aeration tank. When the quantity of MLSS decreased, additional RAS was added without changing the volume of liquor in the aeration tank. The health and population of microorganisms in the activated sludge reactor were checked regularly via microscope. The experiment ran for six weeks and samples were collected daily for fluorescence,BOD_5, COD and total organic carbon (TOC) analyses. Dissolved oxygen concentration and pH were monitored every 30 min in the ASP tank.

In situ measurements

Three portable fluorescence instruments were installed and left unattended at the WwTP

final effluent discharge point, before the discharge to the river. Specifically, these were two submersible probes (Cyclops 7, Turner Designs; EXO1 sonde, YSI Xylem) and a cuvette-based (DuoFluor; designed and manufactured at the University of Birmingham) (Bridgeman et al. 2015). The Cyclops 7 and EXO1 were inserted directly into the final effluent channel. Proprietary protective caps were placed over the two submersible sensors and they were not cleaned for the duration of the experiment. The sensors were also secured with ropes to prevent excessive movement caused by the fluid flow.

The cuvette-based DuoFluor device was installed in an adjacent shed for power connection and protection from rainfall (Fig. S2). The final effluent was pumped to the fluorimeter at a flow-rate of 340 mL/min. A mesh covered the pump end tube to prefilter the water and prevent debris from entering the cuvette. However, biofilm growth was observed with time on the cuvette walls and on the tubing. Consequently, the cuvette was washed (10 % nitric acid) and rinsed with de-ionised water on a weekly basis, and the tubing was replaced after two weeks.

The measurement frequency was set at 15 min for all instruments. Cyclops 7 was initially set up to measure every 30 min, however the number of data points was insufficient to obtain an adequate assessment of water quality fluctuations. No problems occurred with the submersible devices. However, operation of the DuoFluor ceased one week before the end of the experiment due to power failure.

Measurements

Fluorescence peaks

This study focused on specific fluorescence components, assigned to spectral regions T (λ_{ex}/ λ_{em} - ~280 nm / 350 nm) and C (λ_{ex}/ λ_{em} - ~ 330 nm / 425 nm), which can be used to assess the quality of wastewater (Carstea et al. 2016). Peak T is generally associated with living and dead cellular material and their exudates and indicates microbial activity

(Bridgeman et al. 2013). Peak T is also widely associated with material derived from anthropogenic activities (Yu et al. 2014). Several fluorophores could contribute to these regions (Carstea et al. 2016; Coble et al. 2014). Considering the variety of wastewater discharges received by the WwTP and the wavelengths used by the devices, the following components could fluoresce in the peak T region: lignins, aromatic hydrocarbons and indoles originating from domestic waste (partially degraded foods, undigested dietary fibre, toilet paper, proteins and peptides), petrochemical, pharmaceutical and paper industries. Peak C is defined as reduced quinone-like and was identified in OM from a wide variety of aquatic systems, especially those dominated by terrestrial and microbial inputs (Ishii and Boyer 2012). Potential contributors to the fluorescence of peak C could be: lignin breakdown products, quinones, flavonoids, humic acids and fluorescent whitening agents (FWAs) originating from municipal wastewater (food, plants, microbes, fungi, laundry detergents, sanitary products, toilet paper and tissues) and paper making industry (Carstea et al. 2016). In a recent study, it was shown that the removal rates of peaks T and C correlated with the removal of pharmaceuticals, such as gemfibrozil, ibuprofen and naproxil, and with personal care products, such as triclosan or caffeine (Sgroi et al. 2016). Thus, the exact composition of fluorophores cannot be determined by the measurement of peaks T and C, however, these peaks are highly effective in showing the removal of wastewater OM. Apart from these two peaks, the common fluorescence regions reported for FWAs, at excitation wavelength 370 nm and 400 nm (Coble et al. 2014), were also considered, due to the proximity of EXO1 excitation wavelength to one of the FWAs peaks. Past studies (Assaad et al. 2014; Chandler and Lerner 2015; Graham et al. 2015), proposed FWAs as indicators of human faecal contamination, sewer misconnections and landfill leachates.

Fluorescence measurements

Fluorescence was measured with three portable fluorimeters. Cyclops 7 measures the

fluorescence intensity at the excitation / emission wavelengths of 285 nm / 350 ± 55 nm, with a limit detection range of 3 ppb to 5,000 ppb tryptophan standard. EXO1 sonde houses three sensors: fDOM (fluorescence dissolved OM), conductivity/temperature and pH. The fDOM sensor records at 365 ± 5 / 480 ± 4 nm (excitation / emission wavelength pair). The detection range is 0 ppb - 300 ppb quinine sulphate units. DuoFluor is capable of detecting fluorescence in real time at 280/350 nm (Peak T) with minimum limit of detection 1.5 ppb of L-tryptophan and at 330/425 nm (Peak C) with minimum limit of detection 1.5 ppb of quinine sulphate. The linearity between the portable devices and a benchtop spectrofluorimeter (Varian Cary Eclipse) was checked with a series of dilutions of L-tryptophan and quinine sulphate standards (Fig. S3). L-tryptophan solutions were varied between 50 ppb and 250 ppb, while quinine sulphate was prepared in concentrations of 10 ppb to 700 ppb. The linearity of the EXO1 was checked up to 400 ppb of quinine sulphate, as recommended by the manufacturer. R^2 values exceeded 0.98 for all instruments.

Excitation-emission matrices were produced using the benchtop spectrofluorimeter: by scanning excitation wavelengths from 200 to 400 nm in 5 nm steps, and detecting the emitted fluorescence in 2 nm steps between 280 and 500 nm. Excitation and emission slit widths were set to 5 nm. Instrument stability was checked by recording the Raman values (at excitation wavelength 348 nm and emission wavelength 395 nm) before each set of measurements. The average Raman value was 9.94 a.u. with a standard deviation of 0.24. The fluorescence peaks were extracted using the peak-picking method, in accordance with previous studies (Coble et al. 2014).

Ancillary analyses

Rainfall, temperature, total phosphorus, iron, ammonia and suspended solids were measured daily on-site at the WwTP outfall. In addition, samples were collected twice a week for BOD_5, COD, TOC, nitrate and turbidity (Table S1). Low values were observed for all

parameters, indicating effective treatment of the wastewater. BOD_5 was measured based on the standard method for wastewater testing using a HQ40d portable meter (Hach) with an IntelliCAL LBOD101 LDO probe. The accuracy of the BOD_5 measurements was checked using a 300 mg/L glucose-glutamic acid standard, and a coefficient of variation of 3.6 % was observed. COD and nitrate were measured using a DR890 Hach colorimeter, following standard procedures: *viz.* Reactor Digestion Method (USEPA) for COD, and Chromophoric Acid Method (high range, Test 'N Tube) for water and wastewater for nitrate. Turbidity was recorded using a Hach 2100N turbidimeter. TOC measurements were undertaken using a Shimadzu TOC-Vcpn analyser, using the non-purgeable organic carbon determination method.

Results and discussion

Laboratory scale ASP

Before the *in-situ* study, a laboratory-based experiment was undertaken replicating the ASP to establish the relationship with BOD and to determine the potential of using fluorescence spectroscopy for real-time measurements. WwTPs measure BOD on a daily basis; however, a qualitative method is used, which provides ranges of BOD values and the result cannot be compared with fluorescence intensity. The regulatory 5-day BOD test is performed only once per month. Therefore, the laboratory scale ASP was designed to identify this fluorescence/BOD relationship.

Figure 1 shows the fluorescence intensity of peaks T and C measured with the benchtop fluorimeter plotted against BOD. The Kendall correlation coefficients with BOD_5 are: 0.71 ($p<0.001$ – 2-tailed test of significance, N=87) for peak T; and 0.43 ($p<0.001$ – 2-tailed test of significance, N=87) for peak C. The correlation between BOD and fluorescence is challenging to identify at low BOD concentrations (Hudson et al. 2008), thus the values quoted above were determined using a combination of data from final effluent and settled

sewage samples. An improved correlation was observed for BOD with peak T compared to the peak C/BOD relationship was reported in other studies (Bridgeman et al. 2013; Hudson et al. 2007). The various types of fluorophores that contribute to the peaks T and C fluorescence region explain the difference in correlation values. In addition, Reynolds (2002) found that peak T is more representative for the biodegradable organic matter than peak C. Considering the strong correlation between peak T and BOD, obtained in this study, and the relationship reported in other studies (Bridgeman et al. 2013; Carstea et al. 2016; Coble et al. 2014; Hudson et al. 2008), it is clear that peak T fluorescence can detect some of the components measured with BOD. Furthermore, fluorescence spectroscopy provides more information on the nature of OM than the BOD test does and may be used as an independent indicator test for the presence of bioavailable OM (Hudson et al. 2008).

Similar relationships were obtained between fluorescence and COD and TOC. The Kendall correlation coefficients with COD are: 0.72 ($p<0.001$ – 2-tailed test of significance, N=87) for peak T; and 0.44 ($p<0.001$ – 2-tailed test of significance, N=87) for peak C. While, the Kendall correlation coefficients with TOC are: 0.82 ($p<0.001$ – 2-tailed test of significance, N=81) for peak T; and 0.49 ($p<0.001$ – 2-tailed test of significance, N=81) for peak C. The good correlation between peak T and TOC may be attributed to the sugars and lignin (Baker 2002) degraded from sanitary products. However, the relationship between fluorescence peaks and BOD, TOC and COD varies depending on the ratio of fluorescent to non-fluorescent OM in a sample (Henderson et al. 2009).

In situ measurements

Peaks T and C data provided by the 3 devices are shown in Figure 2. Kendall correlation analysis showed an association between EXO1 data and DuoFluor peaks T and C (R^2=0.49 & 0.48, $p<0.001$), while Cyclops 7 data presented a slight correlation with the DuoFluor peak T (R^2=0.28, $p<0.001$) (Table 1). The analysis also revealed that the EXO1 and

the DuoFluor data correlated with peaks T, C and FWAs measured with the Varian benchtop spectrofluorimeter. The variation in correlation coefficients might be explained by the differences in excitation and emission wavelengths used by the devices. For instance, the EXO1 excitation wavelength is closer to the optical region of FWAs, compared to the region where peak C is generally reported (Coble et al. 2014), and compared to the peak C excitation wavelengths measured with the DuoFluor and Varian Cary Eclipse. In addition, the correlations with Varian Cary Eclipse data were established using a small sample size (N=8), a larger dataset being needed to obtain statistically significant correlations. However, the results are sufficient to provide an indication of devices potential to measure peaks T and C *in situ*.

During the experiment, the DuoFluor system recorded a constant decrease in peak C fluorescence intensity (Fig. 2D) due to biofilm formation on the cuvette. Regular cuvette cleaning (twice per week) was required to ensure adequate DuoFluor fluorescence results. The EXO1 and the Cyclops 7 sensors were not cleaned during the entire experiment and no substantial reduction in fluorescence intensity was observed. However, further studies are needed to test the time span until fouling interferes with the fluorescence signal. This experiment shows that submersible instruments are more practical at WwTPs. The advantages of needing less frequent cleaning (no cleaning for at least 1 month) and being battery powered make them preferable for effluent monitoring. Fluorescence data were not corrected for thermal quenching as little impact was expected for a decrease of 0.5^0 C from day to night and of 3^0 C change over the entire period (Fig. S4A). Based on previous work (Carstea et al. 2014), it is estimated that the fluorescence intensity would increase by 0.3 % for a decrease in temperature of 0.5^0 C and by 2.6 % for a 3^0 C temperature change. Temperature correction may be needed in areas with high seasonal variation. Inner filter effect is also known to impact the fluorescence measurements. However, Henderson et al. (Henderson et al. 2009)

showed that the inner filter effect is unlikely to occur in surface and wastewater samples with a TOC concentration below 25 mg/l. The final effluent TOC concentrations measured within the current experiment varied between 6.29 mg/L and 9.28 mg/L. Moreover, the same samples showed absorbance values below 0.20 at 254 nm, this being the threshold recommended by Aiken (Coble et al. 2014) for optically dilute samples. Metal ions have been shown to affect the fluorescence intensity and peak position of OM components (Coble et al. 2014). The average iron concentration measured at the WwTP final effluent discharge point was 0.30 mg/L (Table 2). Poulin et al. (Poulin et al. 2014) found that an iron:organic carbon ratio of 0.3 would reduce the fluorescence intensity between 7 % and 23 % depending on the type of water sample. In this study, an average value of 0.03 for the iron:organic carbon ratio was observed. Suspended solids have been shown to influence the results from in situ fluorimeters (Coble et al. 2014). However, Belzile et al. (2006) found a strong correlation between a submersible fluorimeter and a benchtop spectrofluorimeter, at unfiltered samples with suspended solids concentrations below 35 mg/L. In the current study, the effluent suspended solids concentrations varied from 4.5 mg/L to 20.7 mg/L. Filtration, which would reduce the quantity of suspended solids, may also contaminate the sample and remove a large fraction of fluorescent components that are found in particulate or colloidal form (Coble et al. 2014). Furthermore, one aim of this study was to test the robustness of fluorescence spectroscopy to monitor effluent quality without major intervention during or after measurement. For this purpose, a qualitative analysis of effluent OM, i.e. without correction for inner filter effect or extensive calibration, was sufficient to detect changes in effluent water quality.

Peaks T and C displayed a diurnal variation with a cycle of approximately 12 h, the highest intensity being recorded around midnight and the lowest intensity at noon (Fig. 2). During dry weather days, peak T displayed a decrease in fluorescence intensity of < 9 % for

the Cyclops 7 and 16 % for the DuoFluor between midnight and noon, while peak C decreased by < 10 % for the EXO1 sensor and 17 % for the DuoFluor over the same period. The diurnal variation in fluorescence intensity was consistent with the changes in effluent flow rate, conductivity and pH (Fig. S4 (B) and (C), and Fig. S5). However, fluorescence intensity was not directly proportional to the degree of increase in flow rate. The effluent flow rate presented 2 peaks, every day, of almost equal intensity (Fig. S5). We also observed two peaks in the fluorescence data; the first peak being recorded at midnight and the second peak at approximately 2 pm (Fig. 3). This 2 pm peak was substantially lower in intensity compared with the midnight peak, although high flow rate was recorded. It is concluded that these midnight and 2 pm peaks correspond to intensive household water use during the mornings and evenings. Considering the total wastewater retention time within the WwTP from inlet to discharge point (12-16h) and the additional retention time in the sewerage network from household to the WwTP, it is believed that the high values of peaks T and C observed at midnight correspond to the previous day morning high wastewater input, while the 2 pm peak represents the previous evening water usage.

Several rainfall periods, of different intensity and duration, were recorded during the real-time experiment (Table 1). We divided the precipitation days into 4 events: event I – 13th to 14th of August; event II – 19th of August; event III – 23rd to 27th of August; event IV – 30th of August to 3rd of September. The WwTP is served by a combined sewerage system and therefore rainfall increases the influent flow and modifies the properties of the influent affecting process performance and effluent quality (Wilén et al. 2006). Therefore, it is believed that the amount and frequency of precipitation affects most of the measured water quality parameters, depending on the catchment and sewerage system. Rain events were seen to trigger high ammonia and iron values (Table 2). Precipitation also increased the concentration of total phosphorus; the highest value being recorded during or after the first

300 day of the rain event. Conductivity and pH decreased after each rain event, depending on the
301 intensity of the event (Fig. S4). Conductivity showed a significant decrease after events I and
302 IV, while pH was the parameter least affected by precipitation.

303 A decrease in fluorescence intensity was observed one day after the beginning of each
304 precipitation event (Fig. 2). Precipitation events I and IV generated the greatest decrease in
305 Peak C (32 % & 42 % respectively) measured using the EXO1. Cyclops 7 recorded Peak T
306 reductions of 25 % (event I) and 28 % (event IV). DuoFluor measured a 26 % decrease in
307 peak C and 25 % in peak T following event I. The full impact of event IV was not assessed
308 with the DuoFluor due to data loss following a power outage at the WwTP. However, the
309 same effect is observed on peaks T and C after the other rain events. Overall, the decrease in
310 fluorescence intensity is consistent with the quantity of rain per event. After each rain event
311 the fluorescence intensity increased progressively until the next rainfall. Previous studies on
312 urban river monitoring (Carstea et al. 2009) showed that peaks T and C intensity increased
313 after precipitation events, due to the release of higher quantities of OM with surface runoff
314 compared to the receiving water. Here, a dilution of the wastewater's heavily concentrated
315 OM was observed. Others (Mines et al. 2007) also reported a dilution effect, reflected in a
316 decrease in BOD values. Since BOD correlates with fluorescence (Bridgeman et al. 2013), a
317 rainfall-generated decrease in fluorescence intensity is anticipated.

318 In addition to the daily variation and impact from precipitation, two data anomalies
319 were identified on the 24th of August and 3rd of September, both immediately after midnight
320 (Fig. 2– circled with red). These anomalies are most evident from the EXO1 sensor data. The
321 data are higher than the normal daily variation, with or without precipitation, and may be
322 associated with changes in influent quality or treatment processes. The first anomaly is
323 explained by the release of liquor from the sewage sludge facility on the 24th of August at
324 12:00pm. The WwTP managers report that silt is occasionally released with the liquor,

resulting in elevated concentrations of ammonia in the effluent. Unusually high ammonia was recorded at the same time as the high fluorescence intensity (Fig. S5). The high fluorescence intensity during the first anomaly could indicate the production of autochthonous OM from the sewage sludge liquor (Cohen et al. 2014; Riopel et al. 2014), as peak C components increase in the soluble microbial products with increasing retention times (Yu et al. 2015). Also, condensed polymerized humic-like material may form during biodegradation (Saadi et al. 2006). Therefore, liquor may carry large quantities of autochthonous OM, some of it biologically resistant, produced during the long retention times, along the stages of the sewage sludge facility.

The second anomaly (Fig. 2) is a result of the power issues that occurred at the WwTP. On the 3rd of September, low power caused the aeration tank air blowers to fail. Fluorescence data can be used to identify the process failure. The increase of peak C fluorescence from the second anomaly may represent FWAs present in the sewage. Peak C wavelengths coincide with the fluorescence regions of FWAs (Henderson et al. 2009). However, FWAs were also measured in the excitation/emission wavelengths region of 250 nm / 344 nm and 422 nm (Boving et al. 2004). Almost 80 % of FWAs are removed after the biological treatment and these compounds may be used as molecular markers of less effective treatment processes (Hayashi et al. 2002). Therefore, temporary interruption of the ASP tanks would have led to the presence of untreated FWAs, as seen in the second anomaly.

Thus, real-time, *in situ* analysis demonstrated the ruggedness of fluorescence spectroscopy and the ability to detect minor changes in effluent quality. Fluorescence spectroscopy could be used to identify underperformance issues, albeit with a time lag between the failure and the feedback information. However, fluorescence spectroscopy still represents a fast and effective control method, and a reliable alternative to BOD. The benefits of improved treatment control via fluorescence spectroscopy go beyond CO_2 reductions and

climate change mitigation, as they will also facilitate environmental improvements, reduce operating costs and improve the financial performance of the global wastewater industry.

Conclusions

- This study reported the first real-time monitoring of effluent wastewater using fluorescence spectroscopy. Results show that fluorescence spectroscopy is a robust technique for monitoring changes in effluent quality. It also shows that portable devices can run continuously, for 1 month, without any cleaning procedure in the case of submersible systems (or with limited regular cleaning for cuvette-based fluorimeters). Further studies are needed to test the time span until fouling interferes with the fluorescence signal. In addition, multiple sites should be considered in future studies to account various peculiarities of wastewater input.
- Fluorescence peaks T and C showed that OM varied diurnally depending on the flow rate. Precipitation decreased the fluorescence intensity of both peaks due to dilution of wastewater with runoff. The degree of decrease in fluorescence intensity was found to be proportional to the quantity of rainfall.
- 15 min measurement frequency yielded sufficient data to obtain a detailed assessment of daily variation, precipitation impact on influent quality and treatment process.
- A qualitative analysis of effluent OM, i.e. without correction for inner filter effect or extensive calibration can detect changes in effluent water quality. However, temperature correction may be needed in areas with high seasonal variation. Inner filter effect correction may be required when quantitative measurements are needed.
- Submersible instruments proved to be a more practical tool for *in situ*

measurement compared to the cuvette-based device. The advantages of reduced cleaning frequency (no cleaning for at least 1 month) and battery operation make them preferable for effluent OM monitoring.

- Results showed that fluorescence intensity of peaks T and C was capable of detecting minor changes in influent OM quantity and issues with treatment process. The substantial impact on peak C fluorescence intensity with changes in the system was attributed to the input of autochthonous OM from sewage sludge liquor and the presence of untreated FWAs. Although the variation in fluorescence was more clearly observed at peak C compared to peak T, it is recommended that both peaks are monitored due to variations in wastewater composition.

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Supplemental Data

Figs. S1-S5 and Table S1 are available online in the ASCE Library (ascelibrary.org).

References

Ahmad, S. R., and Reynolds, D. M. (1995). "Synchronous fluorescence spectroscopy of wastewater and some potential constituents." *Water Research,* 29(6), 1599–1602.

Assaad, A., Pontvianne, S., and Pons, M. N. (2014). "Photodegradation-based detection of

fluorescent whitening agents in a mountain river." *Chemosphere*, Elsevier Ltd, 100, 27–33.

Baker, A. (2002). "Fluorescence excitation-emission matrix characterization of river waters impacted by a tissue mill effluent." *Environmental Science and Technology*, 36(7), 1377–1382.

Belzile, C., Roesler, C. S., Christensen, J. P., Shakhova, N., and Semiletov, I. (2006). "Fluorescence measured using the WETStar DOM fluorometer as a proxy for dissolved matter absorption." *Estuarine, Coastal and Shelf Science*, 67(3), 441–449.

Bourgeois, W., Burgess, J. E., and Stuetz, R. M. (2001). "On-line monitoring of wastewater quality: A review." *Journal of Chemical Technology and Biotechnology*, 76(4), 337–348.

Boving, T. B., Meritt, D. L., and Boothroyd, J. C. (2004). "Fingerprinting sources of bacterial input into small residential watersheds: Fate of fluorescent whitening agents." *Environmental Geology*, 46(2), 228–232.

Bridgeman, J., Baker, A., Brown, D., and Boxall, J. B. (2015). "Portable LED fluorescence instrumentation for the rapid assessment of potable water quality." *Science of the Total Environment*, Elsevier B.V., 524–525, 338–346.

Bridgeman, J., Baker, A., Carliell-Marquet, C., and Carstea, E. (2013). "Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy." *Environmental Technology*, 34(23), 3069–3077.

Carstea, E. M., Baker, A., Bieroza, M., Reynolds, D. M., and Bridgeman, J. (2014). "Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching." *Water Research*, 61, 152–161.

Carstea, E. M., Baker, A., Pavelescu, G., and Boomer, I. (2009). "Continuous fluorescence assessment of organic matter variability on the Bournbrook River, Birmingham, UK." *Hydrological Processes*, 23(13), 1937–1946.

425 Carstea, E. M., Bridgeman, J., Baker, A., and Reynolds, D. M. (2016). “Fluorescence
426 spectroscopy for wastewater monitoring: A review.” *Water Research*, 95, 206–219.
427 Chandler, D. M., and Lerner, D. N. (2015). “A low cost method to detect polluted surface
428 water outfalls and misconnected drainage.” *Water and Environment Journal*, 29(2), 202–
429 206.
430 Coble, P. G., Green, S. A., Blough, N. V, and Gagosian, R. B. (1990). “Characterization of
431 dissolved organic matter in the Black Sea by fluorescence spectroscopy.” *Nature*,
432 348(6300), 432–435.
433 Coble, P. G., Spencer, R. G. M., Baker, A., and Reynolds, D. M. (2014). “Aquatic Organic
434 Matter Fluorescence.” *Aquatic Organic Matter Fluorescence*, P. G. Coble, J. Lead, A.
435 Baker, D. M. Reynolds, and R. G. M. Spencer, eds., Cambridge University Press, New
436 York, 75–125.
437 Cohen, E., Levy, G. J., and Borisover, M. (2014). “Fluorescent components of organic matter
438 in wastewater: Efficacy and selectivity of the water treatment.” *Water Research*, Elsevier
439 Ltd, 55, 323–334.
440 Environmental Knowledge Transfer Network. (2008). *Energy Efficient Water and*
441 *Wastewater Treatment*.
442 Galinha, C. F., Carvalho, G., Portugal, C. A. M., Guglielmi, G., Oliveira, R., Crespo, J. G.,
443 and Reis, M. A. M. (2011). “Real-time monitoring of membrane bioreactors with 2D-
444 fluorescence data and statistically based models.” *Water Science and Technology*, 63(7),
445 1381–1388.
446 Graham, P. W., Baker, A., Andersen, M. S., and Acworth, I. (2015). “Field Measurement of
447 Fluorescent Dissolved Organic Material as a Means of Early Detection of Leachate
448 Plumes.” *Water, Air, and Soil Pollution*, 226(7).
449 Hayashi, Y., Managaki, S., and Takada, H. (2002). “Fluorescent whitening agents in Tokyo

450 Bay and adjacent rivers: Their application as anthropogenic molecular markers in coastal
451 environments." *Environmental Science and Technology*, 36(16), 3556–3563.
452 Henderson, R. K., Baker, A., Murphy, K. R., Hambly, A., Stuetz, R. M., and Khan, S. J.
453 (2009). "Fluorescence as a potential monitoring tool for recycled water systems: A
454 review." *Water Research*, Elsevier Ltd, 43(4), 863–881.
455 Hudson, N., Baker, A., and Reynolds, D. (2007). "Fluorescence analysis of dissolved organic
456 matter in natural, waste and polluted waters—a review." *River Research and*
457 *Applications*, 23(6), 631–649.
458 Hudson, N., Baker, A., Ward, D., Reynolds, D. M., Brunsdon, C., Carliell-Marquet, C., and
459 Browning, S. (2008). "Can fluorescence spectrometry be used as a surrogate for the
460 Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from
461 South West England." *Science of the Total Environment*, 391(1), 149–158.
462 Ishii, S. K. L., and Boyer, T. H. (2012). "Behavior of reoccurring parafac components in
463 fluorescent dissolved organic matter in natural and engineered systems: A critical
464 review." *Environmental Science and Technology*, 46(4), 2006–2017.
465 Jouanneau, S., Recoules, L., Durand, M. J., Boukabache, A., Picot, V., Primault, Y., Lakel,
466 A., Sengelin, M., Barillon, B., and Thouand, G. (2014). "ScienceDirect Methods for
467 assessing biochemical oxygen demand (BOD): A review." *Water Research*, Elsevier
468 Ltd, 49, 62–82.
469 Mines, R. O., Lackey, L. W., and Behrend, G. H. (2007). "The Impact of Rainfall on Flows
470 and Loadings at Georgia's Wastewater Treatment Plants." *Water, Air, and Soil*
471 *Pollution*, 179(1), 135–157.
472 Murphy, K. R., Hambly, A., Singh, S., Henderson, R. K., Baker, A., Stuetz, R., and Khan, S.
473 J. (2011). "Organic matter fluorescence in municipal water recycling schemes: Toward a
474 unified PARAFAC model." *Environmental Science and Technology*, 45(7), 2909–2916.

Ou, H. S., Wei, C. H., Mo, C. H., Wu, H. Z., Ren, Y., and Feng, C. H. (2014). "Novel insights into anoxic/aerobic1/aerobic2 biological fluidized-bed system for coke wastewater treatment by fluorescence excitation-emission matrix spectra coupled with parallel factor analysis." *Chemosphere*, Elsevier Ltd, 113, 158–164.

Poulin, B. A., Ryan, J. N., and Aiken, G. R. (2014). "Effects of iron on optical properties of dissolved organic matter." *Environmental Science and Technology*, 48(17), 10098–10106.

Reynolds, D. M. (2002). "The differentiation of biodegradable and non-biodegradable dissolved organic matter in wastewaters using fluorescence spectroscopy." *Journal of Chemical Technology and Biotechnology*, 77(8), 965–972.

Riopel, R., Caron, F., and Siemann, S. (2014). "Fluorescence Characterization of Natural Organic Matter at a Northern Ontario Wastewater Treatment Plant." *Water, Air, & Soil Pollution*, 225(9), 2126.

Saadi, I., Borisover, M., Armon, R., and Laor, Y. (2006). "Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements." *Chemosphere*, 63(3), 530–539.

Sgroi, M., Roccaro, P., Korshin, G. V., Greco, V., Sciuto, S., Anumol, T., Snyder, S. A., and Vagliasindi, F. G. A. (2016). "Use of fluorescence EEM to monitor the removal of emerging contaminants in full scale wastewater treatment plants." *Journal of Hazardous Materials*.

Singh, S., Henderson, R. K., Baker, A., Stuetz, R. M., and Khan, S. J. (2012). "Characterisation of reverse osmosis permeates from municipal recycled water systems using fluorescence spectroscopy: Implications for integrity monitoring." *Journal of Membrane Science*, Elsevier, 421–422, 180–189.

Singh, S., Henderson, R. K., Baker, A., Stuetz, R. M., and Khan, S. J. (2015). "Online

fluorescence monitoring of RO fouling and integrity: analysis of two contrasting recycled water schemes." *Environ. Sci.: Water Res. Technol.*, Royal Society of Chemistry, 1, 689–698.

Tartakovsky, B., Lishman, L. A., and Legge, R. L. (1996). "Application of multi-wavelength fluorometry for monitoring wastewater treatment process dynamics." *Water Research*, 30(12), 2941–2948.

Wilén, B.-M., Lumley, D., Mattsson, A., and Mino, T. (2006). "Rain events and their effect on effluent quality studied at a full scale activated sludge treatment plant." *Water Science and Technology*, 54(10), 201–208.

Yang, L., Han, D. H., Lee, B. M., and Hur, J. (2015). "Characterizing treated wastewaters of different industries using clustered fluorescence EEM-PARAFAC and FT-IR spectroscopy: Implications for downstream impact and source identification." *Chemosphere*, Elsevier Ltd, 127, 222–228.

Yu, H., Qu, F., Sun, L., Liang, H., Han, Z., Chang, H., Shao, S., and Li, G. (2015). "Relationship between soluble microbial products (SMP) and effluent organic matter (EfOM): Characterized by fluorescence excitation emission matrix coupled with parallel factor analysis." *Chemosphere*, Elsevier Ltd, 121, 101–109.

Yu, H., Song, Y., Liu, R., Pan, H., Xiang, L., and Qian, F. (2014). "Identifying changes in dissolved organic matter content and characteristics by fluorescence spectroscopy coupled with self-organizing map and classification and regression tree analysis during wastewater treatment." *Chemosphere*, Elsevier Ltd, 113, 79–86.

Figure captions

Fig. 1. Relationship between BOD and fluorescence. (A) peak T and (B) peak C (N=87).

Fig. 2. In situ fluorescence measurements. Peak T measured with (A) Cyclops 7 and (B) DuoFluor. Peak C measured with (C) EXO1 and (D) DuoFluor. Rainfall events I-IV are marked with blue and anomalies are circled with red. The DuoFluor stopped recording during rain event IV due to a power failure at the WwTP. The large differences in the fluorescence intensity observed at graph (D) for the dates Aug 17, Aug 20, Aug 24 and Aug 28 were caused by cuvette cleaning on the DuoFluor.

Fig. 3. Examples of daily fluorescence variation for the 3 portable devices. (A) peak T fluorescence and (B) peak C fluorescence. The 2 pm peak is marked with a blue square.

533 **Table 1.** Kendall Correlation Between the Data from Portable Devices and Varian Benchtop

534 Spectrofluorimeter.

Device		Cyclops 7	EXO1	DuoFluor		Varian			
				Peak T	Peak C	Peak T	Peak C	FWA 370	FWA 400
Cyclops 7		1	**0.19**	**0.28**	0.02	0.21	0.21	0.29	0.36
EXO1		-	1	**0.49**	**0.48**	0.64	0.64	0.86	**0.93**
DuoFluor	Peak T	-	-	1	-	0.33	0.20	0.47	0.47
	Peak C	-	-	-	1	0.60	0.47	0.73	0.73

535 Note: Correlation coefficients in bold have p values below 0.001 (p-values < 0.001 are considered significant).

536 **Table 2.** Standard Parameters Measured by the WwTP.

Date	Precipitation (mm)	Temperature (^{0}C)	Total Phosphorus (mg/L)	Iron (mg/L)	Ammonia (mg/L)
10.08	0.0	19.5	0.57	0.31	0.06
11.08	0.0	18.9	0.73	0.29	0.06
12.08	0.0	18.8	0.70	0.33	0.21
13.08	33.0	19.4	0.44	0.34	0.21
14.08	3.0	18.5	0.98	0.49	2.37
15.08	0.0	19.2	0.47	0.35	0.35
16.08	0.0	19.7	0.30	0.28	0.30
17.08	0.0	18.5	0.62	0.28	0.06
18.08	0.0	18.2	0.50	0.33	0.06
19.08	8.0	18.9	0.44	0.37	0.08
20.08	0.0	19.2	0.61	0.33	0.30
21.08	0.0	19.5	0.46	0.25	0.06
22.08	0.0	19.7	0.38	0.35	0.35
23.08	9.0	20.1	0.37	0.46	0.32
24.08	9.0	18.9	0.55	0.27	0.06
25.08	7.0	18.4	0.66	0.30	1.81
26.08	0.5	18.7	0.38	0.26	0.06
27.08	0.5	18.3	0.48	0.25	0.06
28.08	0.0	18.4	0.39	0.21	0.06
29.08	0.0	18.9	0.49	0.24	0.06
30.08	18.0	19.2	0.51	0.17	0.06
31.08	12.0	18.3	0.39	0.25	0.06
01.09	25.0	17.0	0.51	0.20	0.10
02.09	4.0	17.0	0.30	0.19	0.27
03.09	1.0	17.3	0.37	0.23	0.07
04.09	0.0	17.1	0.50	0.40	0.68
05.09	0.0	17.1	0.37	0.25	0.40
06.09	0.0	17.4	0.49	0.24	0.30
07.09	0.0	17.7	0.54	0.34	0.15

537

Supplementary data

Online fluorescence monitoring of effluent quality in wastewater treatment plants

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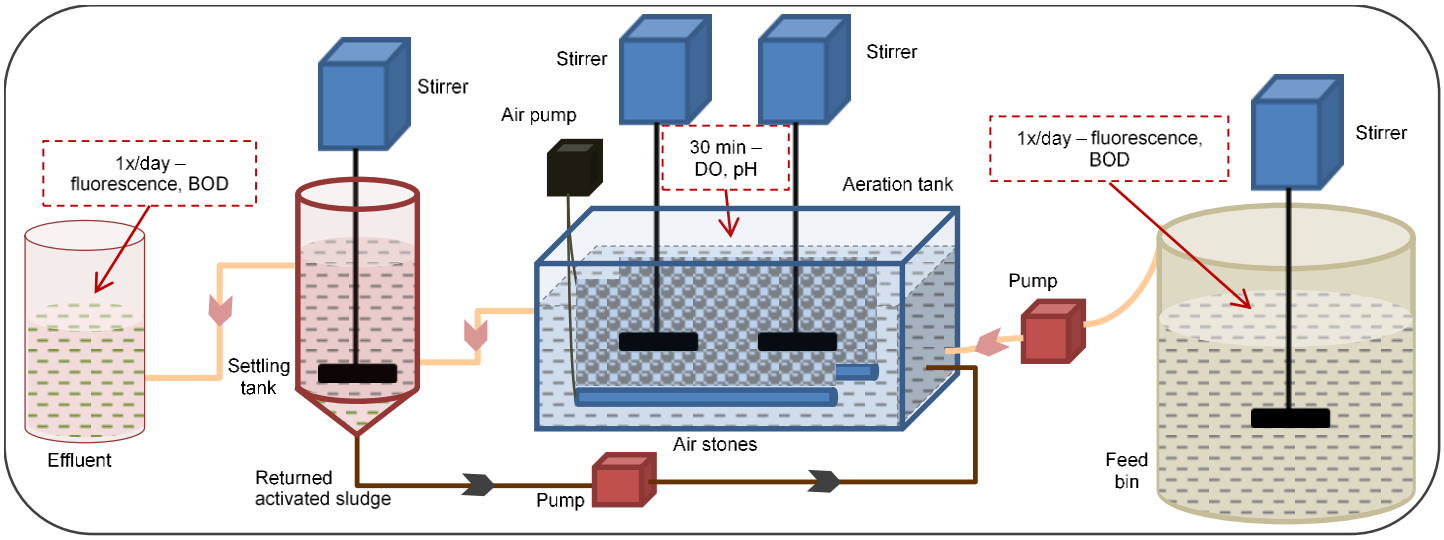

Fig. S1. The setup for the laboratory scale activated sludge process. BOD – biochemical oxygen demand; DO – dissolved oxygen. The orange and black arrows indicate the direction of the flow.

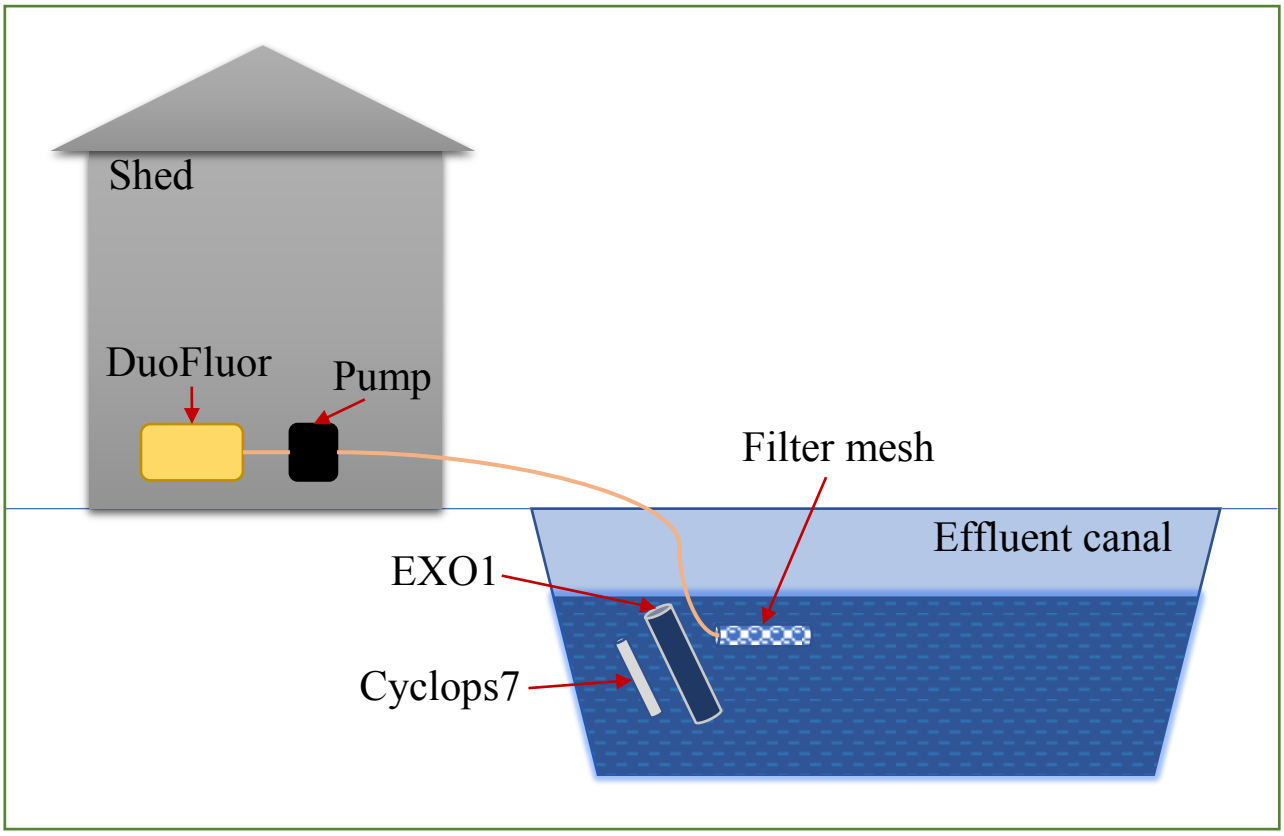

Fig. S2. The setup for the *in-situ* fluorescence measurements. The EXO1 and Cyclops 7 were connected to handheld devices and tightened with ropes to the cover grid above the effluent channel.

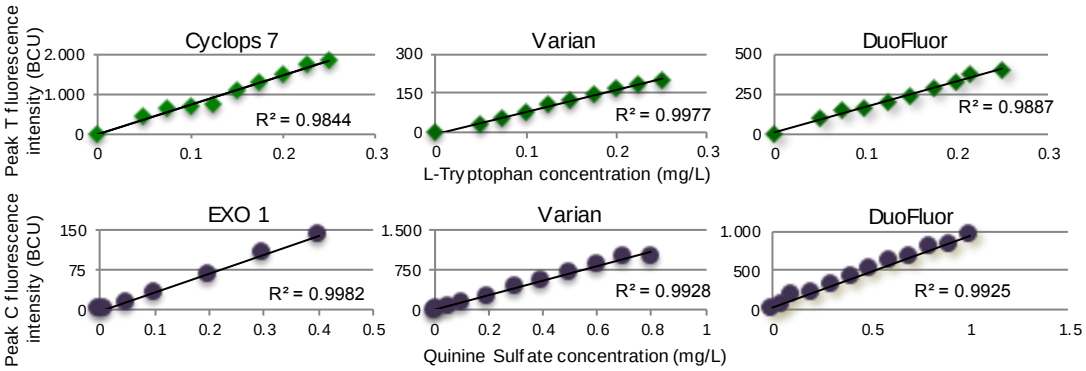

Fig. S3. Linearity check of the three portable devices and comparison with a benchtop spectrofluorimeter. The fluorescence intensity was corrected by extracting the blank spectrum.

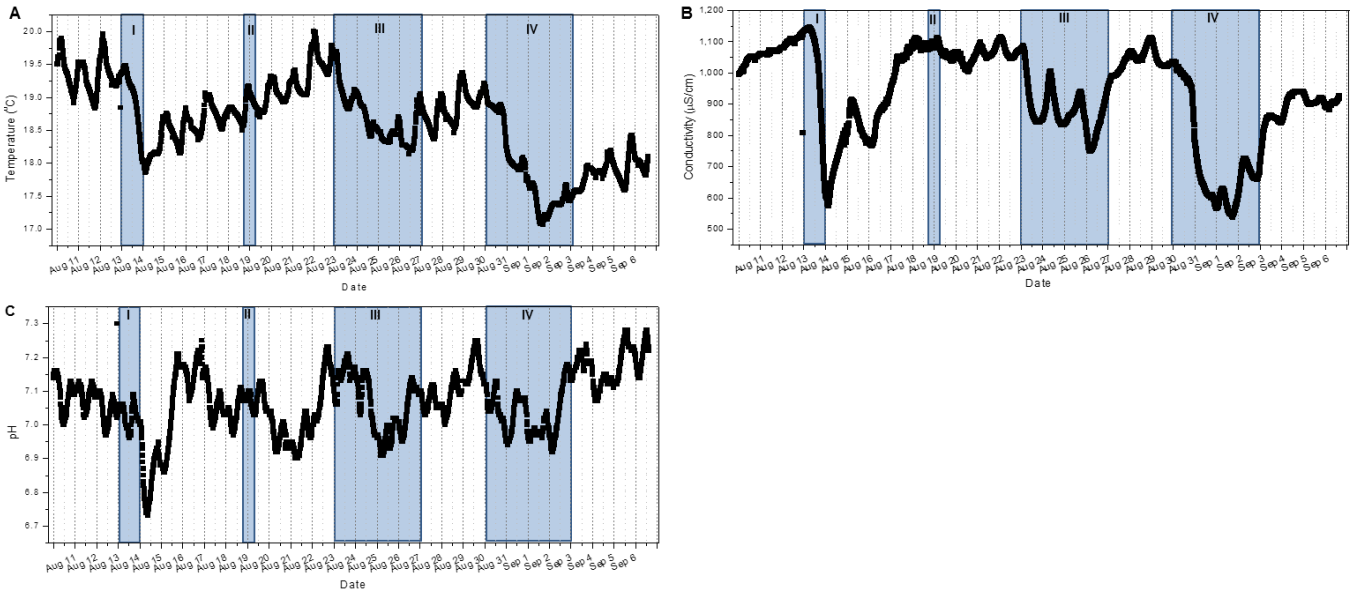

Fig. S4. In situ measurements with the EXO1 sonde. (A) temperature, (B) conductivity and (C) pH.

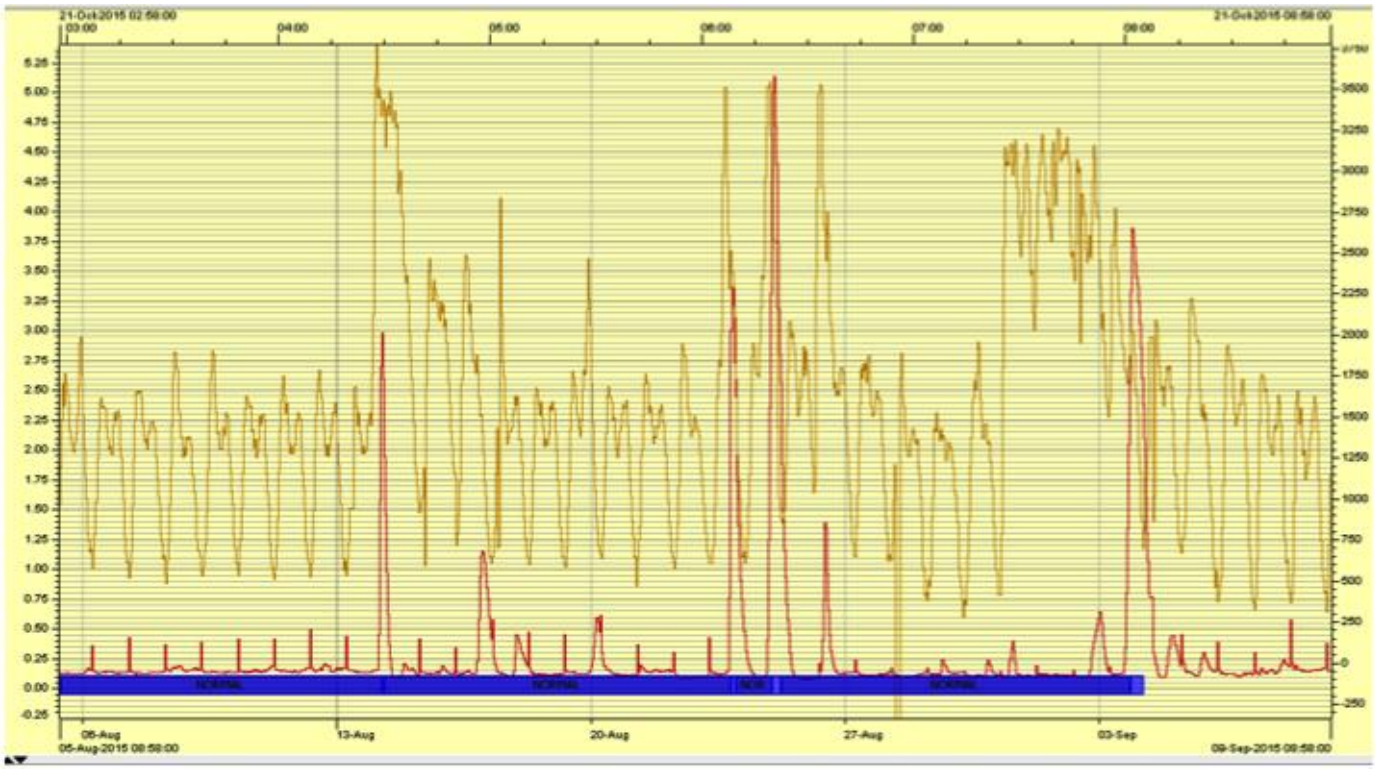

Fig. S5. Flow rate (brown line) and quantity of ammonia (red line) at the effluent. Graph provided by the WwTP.

Table S1. Standard parameters and peak T and peak C fluorescence for grab samples of final effluent. The fluorescence peaks were measured with a benchtop spectrofluorimeter.

Date	BOD	COD	TOC	Nitrates	Turbidity	Fluorescence intensity			
						Peak T	Peak C	FWA 370*	FWA 400*
	(mg/L)				(NTU)	(a.u.)			
13.08.2015	1.6	27	8.9	30.7	N/A	106	178	80	58
17.08.2015	2.9	23	8.5	23.8	2.18	100	166	68	51
20.08.2015	1.9	24	9.3	28.8	1.90	104	189	79	56
25.08.2015	3.0	30	7.9	19.0	1.85	94	148	67	50
28.08.2015	1.7	32	N/A	27.3	1.54	107	168	68	50
01.09.2015	2.7	15	6.3	14.9	2.18	76	129	58	38
03.09.2015	3.0	18	7.2	16.5	2.67	81	148	66	39
07.09.2015	1.7	17	8.5	22.1	1.96	97	159	68	48

* Fluorescence whitening agents' excitation wavelength