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Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy

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Fluorescence spectroscopy was used to characterize municipal wastewater at various stages of treatment in order to understand how its fluorescence signature changes with treatment and how the signal relates to biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The impact of size fractionation on the fluorescence signal was also investigated. Fluorescence measurements were taken for unfiltered and filtered (0.45 and 0.20 μm) samples of crude, settled and secondary treated wastewater (activated sludge and trickling filter), and final effluent. Good correlations were observed for unfiltered, diluted wastewater samples between BOD and fluorescence intensity at excitation 280 nm, emission 350 nm (Peak T1) ($r = 0.92$) and between COD and Peak T1 intensity ($r = 0.85$). The majority of the T1 and T2 signal was found to be derived from the <0.20 μm fraction. Initial results indicate that fluorescence spectroscopy, and changes in Peak T1 intensity in particular, could be used for continuous, real-time wastewater quality assessment and process control of wastewater treatment works.

Keywords: fluorescence spectroscopy; wastewater quality; fractionation; BOD; COD

Introduction

Wastewater analysis

The quality of water and wastewater is generally assessed using a series of physical, chemical and microbiological tests. Traditionally, significant reliance has been placed on the biochemical oxygen demand (BOD) test, and it has been used globally as part of effluent discharge consents at wastewater treatment works (WwTW) since the Royal Commission report of 1912. In simple terms, BOD provides an indication of the oxygen uptake of microorganisms whilst breaking down organic matter present in the water. It is, therefore, a measure of the oxygen depletion potential of a pollutant load in water. It is assessed off-line, typically over a five-day period, and test results are often referred to as BOD_5 results.[1] However, despite its widespread use, the BOD_5 test has severe limitations (Table 1). These have been discussed extensively in the literature (e.g. [2]). In summary, the BOD_5 test has an uncertainty of 15–20% in the results,[2] is limited to a five-day period and so represents only a portion of the total BOD, and is unrepresentative of natural reactions in watercourses.

The chemical oxidation demand (COD) is a test involving chemical oxidation using boiling potassium dichromate and concentrated sulphuric acid to determine the amount of oxygen needed to chemically oxidize the organics present in a (waste)water.[3,4] In comparison to BOD_5, the COD test takes just 2 h and is unaffected by the presence of toxic compounds. However, the COD test yields no information regarding the rate of biodegradation, nor does it distinguish between biodegradable and biologically inert organic matter. Also, the necessity to use chromium, silver and magnesium produces a hazardous waste, the disposal of which can be problematic.

It is clear from the above that both BOD_5 and COD are means by which one can estimate the amount of oxygen required for biodegradation. Used in combination, one can generate an indication of wastewater biodegradability. However, neither test can be used to characterize wastewater for the purposes of real-time process control.

The treatment of wastewater is an energy-intensive operation. The largest energy usage in wastewater treatment is found in the aeration of settled wastewater in the activated sludge process (ASP) which, in isolation, contributes to over half of the energy costs associated with wastewater treatment. Currently, the process is often monitored and controlled by *in situ* dissolved oxygen or ammonia measurements. However, the performance of dissolved oxygen probes is variable and affected by location in the plant, and

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Table 1. Limitations of the 5-day BOD test from [2].

- Five days to generate a result
- Dilution approach reduces substrate and micro-organism concentrations, so decreasing kinetic rates
- Presence of toxic substances is inhibitory to organic waste oxidation by bacteria
- Insensitive at low concentrations
- Imprecise and uncertain (15–20%)
- Labour intensive
- Unsuitable for process control
- Unsuitable for real-time monitoring

the performance of ammonia probes is affected by the hostile environment within which they are required to work. Consequently, water utilities often over-aerate in order to achieve compliance with final effluent standards. A real-time analytical technique could potentially save up to 40% of the energy currently employed in the continuous aeration in ASPs.[5] However, the limitations of BOD_5 and COD render them inadequate for effective and efficient process control.[5] There is, therefore, a clear need for the development of improved process control to unlock the anticipated 40% savings. The benefits of such improved operation and control would include much needed CO_2 reductions, whilst also facilitating environmental improvements, reducing operating costs and improving the financial performance of the global wastewater industry.

The challenge, therefore, is to identify and develop an appropriate technology to characterize wastewater and to improve process control of the ASP (to minimize over-aeration), and wastewater treatment systems in general, so eliminating the additional, unnecessary costs associated with over-treatment. The research reported in this paper explores the use of fluorescence spectroscopy to characterize wastewater at various stages of treatment in order to understand how that character changes with different treatment processes. Specifically, the aims of this work are to (1) identify the fluorescence properties of wastewater through treatment processes, (2) to investigate the impact of particle size fractions on the fluorescence signal and (3) to explore any relationships between fluorescence and conventional laboratory-based characterization techniques (i.e. BOD_5 and COD).

Fluorescence spectroscopy

Fluorescence spectroscopy is an established analytical method used to identify dissolved organic matter (DOM), trace organics and pollutants in marine, surface and groundwaters. Excitation in the ultraviolet and visible light ranges causes organic matter present in a sample of water to fluoresce. The nature and extent of this fluorescence is a function of the fluorophores present. Simultaneous scanning of a range of excitation and emission wavelengths (λ_{ex} and λ_{em} nm) generates an excitation–emission matrix (EEM) within which fluorescent peak intensities can be identified within certain regions. Fluorescence in specific regions may be indicative of different organic matter. For example, in freshwaters, fluorescence in the region [$230 < \lambda_{ex} < 260$ and $400 < \lambda_{em} < 500$ nm] (Peak A) is often attributed to terrestrially derived organic matter. Fluorescence in the region [$300 < \lambda_{ex} < 370$ and $400 < \lambda_{em} < 500$ nm]) (Peaks C and M) is increasingly ascribed to dissolved organic carbon that has been microbially processed. Peaks T1 [$\lambda_{ex} = 275$ and $\lambda_{em} = 340$ nm] and T2 [$225 < \lambda_{ex} < 235$ and $340 < \lambda_{em} < 360$ nm]) are ascribed to tryptophan-like material found within living and dead cellular material and their exudates, and are indicative of microbial activity in a water. For reviews of the use of fluorescence spectroscopy in organic matter characterization see Hudson et al.,[6] Henderson et al.,[7] Bridgeman et al.[8] and Ishii and Boyer.[9] Whilst research into the application of fluorescence spectroscopy has been extensive in some areas (e.g. surface water), investigation into how fluorescence might be applied to wastewater process optimization is comparatively limited; with the majority of published research in this field focusing on treated wastewater effluents (for example, [10]), a single aspect of the treatment process, or application to wastewater recycling.

Bari and Farooq [11] first reported the use of fluorescence in their investigation of the treatment efficiency of potassium ferrate and ozone, in various combinations, for the removal of organic matter from different wastewaters. Ahmad and Reynolds [12] and Reynolds and Ahmad [13] further demonstrated the fluorescence emission spectra of wastewaters using a number of different excitation wavelengths. Reynolds and Ahmad [5] found that Peak T was reduced in wastewater treatment to a greater extent than either Peak A or C . They attributed this to the crude wastewater being anthropogenically derived and hence representing fresher material with higher oxidation potential. From this, Reynolds and Ahmad [5] suggested that fluorescence, and Peak T in particular, could be used as a surrogate for the BOD_5 test. Hudson et al. [10] analysed 469 water samples (246 river samples and 223 effluents, being municipal WwTW final effluents, trade waste discharges and final effluents) for fluorescence and BOD_5. For the river water, results showed Peaks T1 and T2 commonly present in all samples, with T2 always greater than T1. Peaks A and C were also routinely present, with Peak A intensity always exceeding that of Peak C. Unsurprisingly, the Peak T1 and T2 intensities of the wastewater samples were greater than those of the river samples, and, again, T2 was greater than T1, with the former occasionally masking Peak A. Hudson et al. [10] attempted to correlate peak fluorescence intensities with BOD_5. Limitations associated with low concentration BOD analysis eliminated 175 samples from the analysis. However, the remaining data demonstrated strong correlations between T1 and BOD ($r = 0.906$ for all samples, $r = 0.714$ for effluent samples only). There was a strong correlation between T2 and BOD_5

for all samples ($r = 0.848$), but this was reduced for the effluent samples ($r = 0.472$). The reasons for this reduction are unclear. Peaks A and C showed similar responses; for all samples $r = 0.771$ and 0.720 for Peaks A and C respectively; and for the effluent samples $r = 0.341$ and $r = 0.331$ for Peaks A and C respectively. The authors suggested that correlations between Peak T and BOD_5 exist because Peak T is present in a bioavailable substrate or it (Peak T) is produced by microbial action while it is using a bioavailable organic fraction.

More recently, Liu et al. [14] used fluorescence EEMs to assess the performance of submerged membrane bioreactors treating polluted surface water with and without pre-ozonation. The authors used EEM fluorescence spectroscopy to characterize the DOM samples, extracellular polymeric substance samples and membrane foulants. Results showed that pre-ozonation decreased Peaks T1, T2, A and C and that membrane fouling could be effectively mitigated by pre-ozonation.

Hur et al. [15] applied fluorescence spectroscopy to an analysis of refractory dissolved organic matter (RDOM) in wastewater. Considering data from 15 WwTW, the authors found that DOM removal efficiency improved in instances when influent DOM exhibited a lower specific ultra violet absorbance (SUVA, the ratio of UV absorbance at 254 nm to dissolved organic carbon concentration) reduced humic-like fluorescence, and a lower RDOM distribution. The authors concluded that influent characteristics may offer an indication of DOM removal efficiency. However, similar results were not observed for RDOM removal efficiency, where a slight positive correlation was observed, suggesting that refractory organic carbon structures present in the influent sewage may stimulate microbial activity and inhibit the RDOM production during biological treatment.

Quaranta et al. [16] used fluorescence to characterize final effluent organic matter from five Connecticut (USA) municipal WwTW. The results demonstrated that effluent organic matter characteristics differ from terrestrial organic matter that is typically present in small to moderately sized rivers. Fluorescence characterization showed both fluorescence Peaks A and C, as well as Peaks T1, T2 and B, the latter (at emission of ~305 nm) not commonly observed for terrestrial organic matter. Interestingly, although five WwTWs were analysed, and the sizes and treatment processes differed, little variation in effluent organic matter characteristics was observed between the WwTWs, suggesting that the effluent organic matter characteristics are similar, regardless of treatment works design. Esparza-Soto et al. [17] also used fluorescence EEMs to characterize effluent organic matter, in this instance that arising from a lab-scale activated sludge sequencing batch reactor (SBR). Peaks T1, T2 and C were identified in the effluent organic matter which the authors attributed to soluble microbial products generated within the SBRs.

Recent years have seen an expansion of interest in the application of fluorescence to wastewater recycling. Singh et al. [18] used EEM fluorescence spectroscopy to characterize reverse osmosis (RO) permeates from three water recycling plants. The authors found that Peak C and Peak T fluorescence could distinguish intermediate permeates from multiple staged RO treatment processes. Results indicated that fluorescence is a more selective and sensitive method for monitoring the organic composition of RO permeates than established methods and the authors concluded that fluorescence monitoring is a promising technique for sensitive performance monitoring of RO treatment processes.

Recently, Cumberland et al. [19] used a portable light emitting diode (LED) spectrophotometer to establish bacterial numbers in a range of water samples. Fluorescence from uncultured dilutions were detected at a 280 nm excitation/360 nm emission wavelength (T2) and compared with bacteria numbers on the same cultured sample. Cumberland et al. [19] reported good correlations between the Peak T2 response of the portable LED spectrophotometer and total coliforms, *Escherichia coli*, and heterotrophic bacteria (HB) ($r^2 = 0.78$, 0.72 and 0.81, respectively). The authors suggested that the portable spectrophotometer could be applied to establish the quality of drinking water in areas of poor sanitation which may have been subject to faecal contamination, for example, as a result of infrastructure failure.

Whilst earlier work suggests that fluorescence intensity of wastewater reduces with treatment,[13,20] little is known about how the fluorescent properties of wastewater vary between treatment processes (both with and without sample filtration), and also about the robustness of any relationship with peak intensities. The work reported in this paper describes an investigation of these areas in order to demonstrate the potential for fluorescence spectroscopy to be used in the routine analysis of wastewater and treatment works performance.

Materials and methods

Site selection

Wastewater samples were taken from Site A, a 77,000 population equivalent WwTW located in the Worcestershire region of the UK. The WwTW has parallel ASP (treating 60% of the flow) and nitrifying trickling filters (treating the remaining 40% of the flow) following a common primary treatment stage. Secondary treated sludge is then combined and filtered through a tertiary deep bed sand filter before discharge to a watercourse (Figure 1). Average flow through the WwTW is 382 m^3/s, and maximum treatment capacity is 637 m^3/s. Both secondary treatment processes can be dosed with iron salts; the ASP flow being dosed with ferrous chloride, and the trickling filter effluent being dosed with ferric sulphate immediately upstream of the humus tanks. At the time the work reported here was

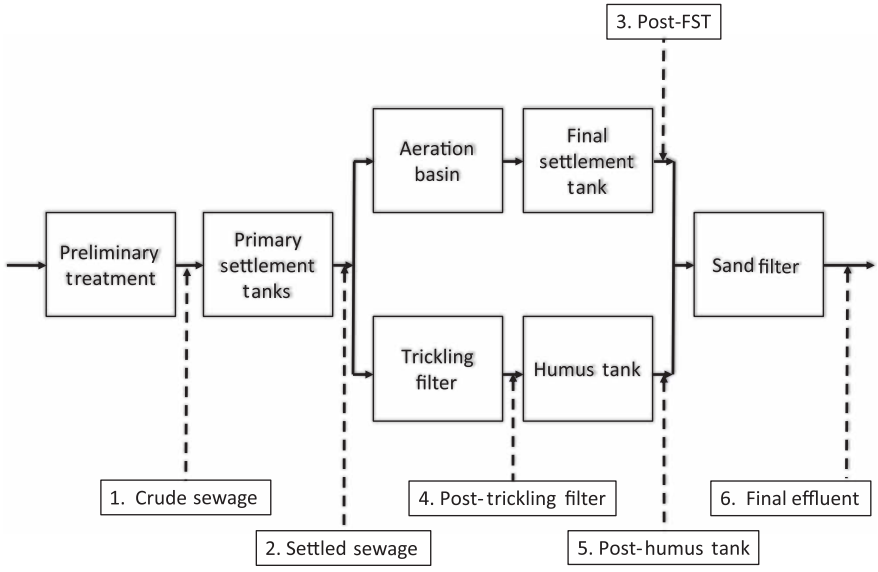

Figure 1. Process flow schematic of WwTW and sampling points.

carried out, both streams were dosed at a constant rate of 35 mg/l/h. Samples for analysis were taken at six locations at the WwTW: (1) Crude wastewater, (2) Settled wastewater (primary settlement tank (PST) effluent), (3) ASP (final settlement tank (FST) effluent), (4) Trickling filter effluent, (5) Humus tank effluent and (6) Final treated effluent. Sampling points are shown in Figure 1. Poor access meant that it was not possible to sample immediately downstream of the aeration basins, precluding any direct comparison of aeration basin and trickling filter performance. However, a comparison of total ASP (aeration basins and FSTs) and combined trickling filter and humus tanks was possible.

Sampling

Samples were taken from each sampling point on eight occasions over a one-month period (29 June, 10, 11, 13, 17, 18, 20, 24 July 2006), and all samples were taken between 0930 and 1015 on each day. The first sample was always taken at the final effluent discharge point (sample point 6), to avoid contamination of subsequent samples. A weighted 1-litre sampling bucket was used for sample collection. At each sampling point, the bucket was immersed in the flow and rinsed twice to remove potential contaminants from the previous sample. The bucket was then filled with (partially-) treated wastewater from just below the surface. The sample was transferred into 1 litre and 50 ml containers. All samples were stored in a sealed cool box and transferred to Severn Trent Laboratories for BOD_5 and COD analyses (1 litre bottles), and to the University of Birmingham for fluorescence analysis (50 ml samples) where they were immediately filtered and fluorescence analysis was undertaken within six hours of sampling. BOD_5 and COD analyses commenced with 24 h of sampling.

Analyses

Samples were filtered using pre-cleaned 0.2 and 0.45 μm Whatman/GMF poly-vinyl-idine-difluoride filters. Fluorescence intensity was measured using a Cary Eclipse Fluorescence Spectrophotometer (Varian, Surrey, UK), 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 and photomultiplier tube voltage to 725 V. To monitor instrument stability, scans of a sealed cell containing deionized water were run and the intensity of the Raman line of water at 348 nm excitation wavelength was recorded. All fluorescence intensities were corrected to a Raman peak intensity of 20 units. Results can be compared with a quinine sulphate standard: 32.5 intensity units is equivalent to 1 quinine sulphate unit (1 $\mu g.l^{-1}$ in 0.1 M H_2SO_4). EEMs were obtained for each unfiltered wastewater sample, and for 0.2 μm, 0.45 μm and unfiltered fractions (to investigate the impact of filtering on fluorescence. It was assumed that 0.45 μm filtration would remove particulate matter which could cause scatter, and 0.2 μm filtration would remove the microbial fraction from each sample. All samples were diluted 10:1 to further reduce any scattering, as well as the potential for inner filter effects. Standard methods HMSO [1,4] were followed for BOD_5 and COD.

Fluorescence peak information was extracted using the peak-picking method, which identifies the position and intensity of the peak from an EEM. The authors have previously considered various multivariate approaches to data analysis [21]: however, peak-picking has been demonstrated to be an appropriate approach for real-time monitoring applications.[18,22–24] It is accepted that alternative approaches exist (e.g. singular value decomposition); however, in view of the authors' previous success with the use

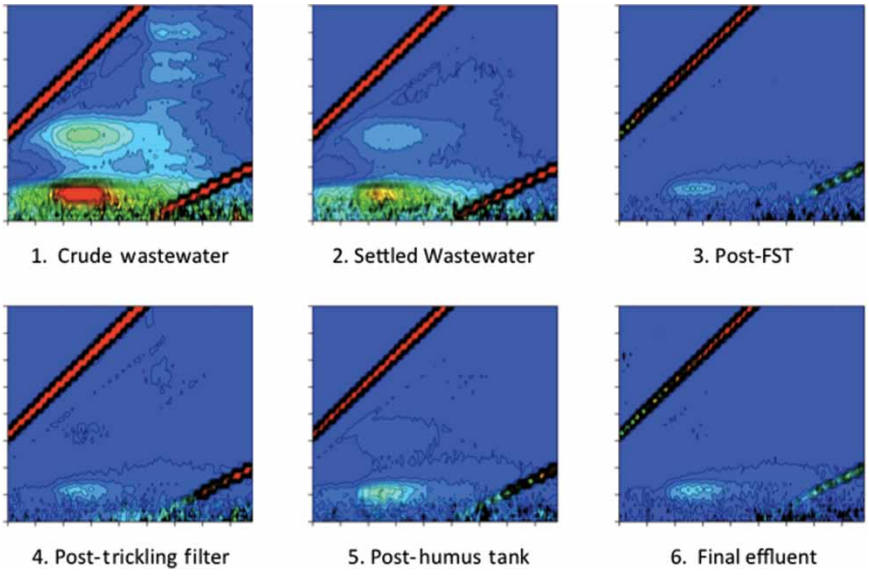

Figure 2. EEMs through the WwTW, 24 July 2006. 1 = crude, 2 = PST effluent, 3 = ASP effluent, 4 = Trickling filter effluent, 5 = Humus tank effluent and 6 = final treated effluent.

of peak-picking for environmental sensing, the decision was taken to adopt this approach here. The intensity and the excitation and emission wavelengths of the peaks were obtained automatically by searching for the maximum fluorescence intensity value and its corresponding emission and excitation wavelength within the following ranges: λ_{ex}: 275–285 nm, λ_{em}: 340–360 nm (Peak T1), λ_{ex}: 225–235 nm, λ_{em}: 340–360 nm (Peak T2), λ_{ex}: 225–235 nm, λ_{em}: 410–440 nm (Peak A) and λ_{ex}: 320–355 nm, λ_{em}: 410–470 nm (Peak C). The ranges were specifically chosen to avoid overlap between peaks signal or with scattering.

Results and discussion

EEMs based on samples taken from various treatment stages on the same day are shown in Figure 2. Figure 2 shows clearly that crude sewage samples show the highest fluorescence intensities demonstrating that these samples contain elevated concentrations of organic matter. From Figure 2 it is clear that peaks exist which indicate the presence of whitening agents (λ_{ex}: 370 nm, λ_{em}: 410 nm), Tryptophan-like 1 and 2 (T1, T2, λ_{ex}: 275 nm, λ_{em}: 340 nm and λ_{ex}: 225–237 nm, λ_{em}: 340–380 nm, respectively), and humic and fulvic-like peaks (A and C, λ_{ex}: 237–260 nm, λ_{em}: 400–500 nm, and λ_{ex}: 300–370 nm, λ_{em}: 400–500 nm, respectively). Tyrosine-like fluorescence (Peaks B1 and B2 λ_{ex}: 225–237 nm, λ_{em}: 309–321 nm, and λ_{ex}: 275 nm, λ_{em}: 310 nm, respectively) cannot be identified from Figure 2. Tyrosine-like peaks are particularly challenging to identify at low concentrations given the proximity of B2 to the water Raman line, and at all concentrations by their quenching by tryptophan, when both tryptophan and tyrosine are present in protein residues. This means that they can be obscured when T1 and T2 fluorescence intensities are high, and this is likely to be the reason for their apparent absence here. Analysing the data from all eight days of analysis, a significant peak (1036–3410 au) was observed in crude wastewater at $\lambda_{ex} = 370$, $\lambda_{em} = 410$ nm for six of the eight days. Fluorescence in this region is indicative of fluorescent whitening agents or detergents in the wastewater. For each occurrence, the peak was found only in the crude wastewater and not beyond, indicating that the dissolved fraction that fluoresces is associated with the particulate fraction which is removed in preliminary treatment or primary sedimentation tanks. For each sample day, Peaks T1 and T2 fluorescence were greater than all other recognized peaks, and were both found to reduce with treatment.

Figures 3 and 4 show the reduction in Peak T1 with treatment through each of the two streams on each sampling day. These figures demonstrate the significant variation in crude wastewater Peak T1 fluorescence (5020–1867 au) and the consistent removal of the fluorophores resulting in a relatively consistent final effluent quality (339–559 au). A similar trend was observed for Peak T2. It is interesting to note that the range of values in crude wastewater narrows considerably after the PSTs. It is possible that data from 24 July represent dry weather flow conditions, so producing concentrated organic matter but increased hydraulic retention time in the PSTs; however, flow analysis was not undertaken at the time to demonstrate this.

Mean crude wastewater T1 and T2 values measured over the sampling period were 2501 and 9152 au respectively. Mean T1 and T2 intensity values of primary settled wastewater were 1713 and 7017, respectively, indicating a 31.5% reduction of Peak T1 and 23.3% reduction of Peak T2 in the PSTs. Figure 5 shows the mean T1 and T2 intensities measured immediately downstream of the FSTs

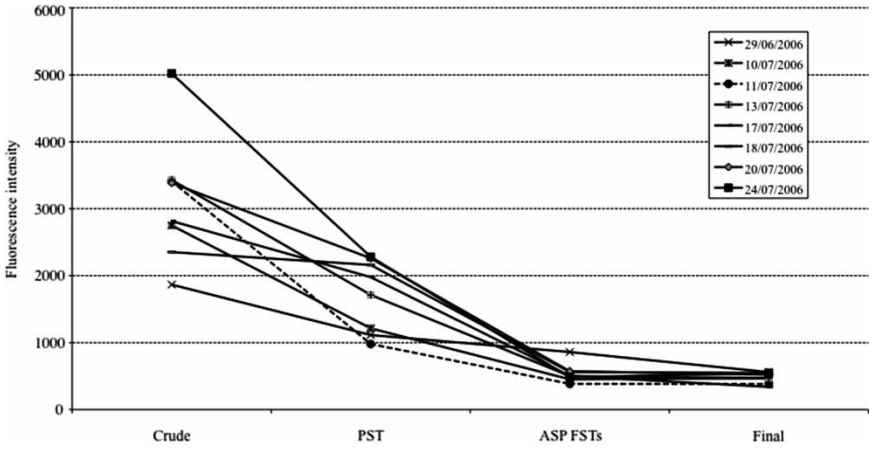

Figure 3. Peak T1 intensity reductions through activated sludge plant works on eight sampling days.

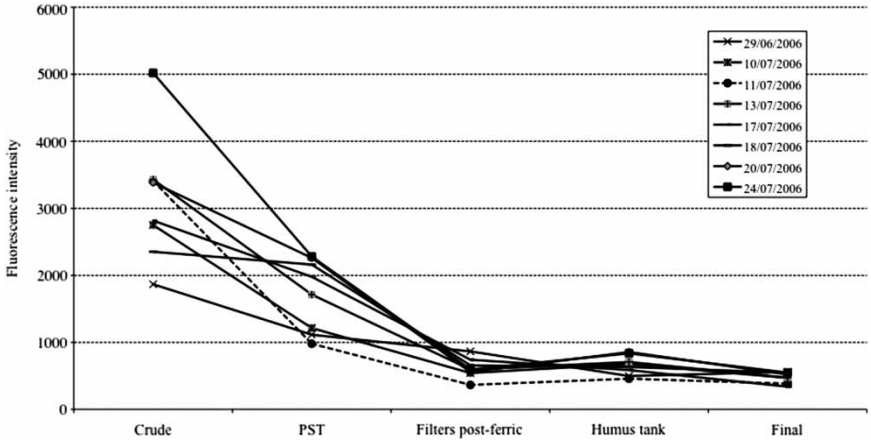

Figure 4. Peak T1 intensity reductions through trickling filter works on eight sampling days.

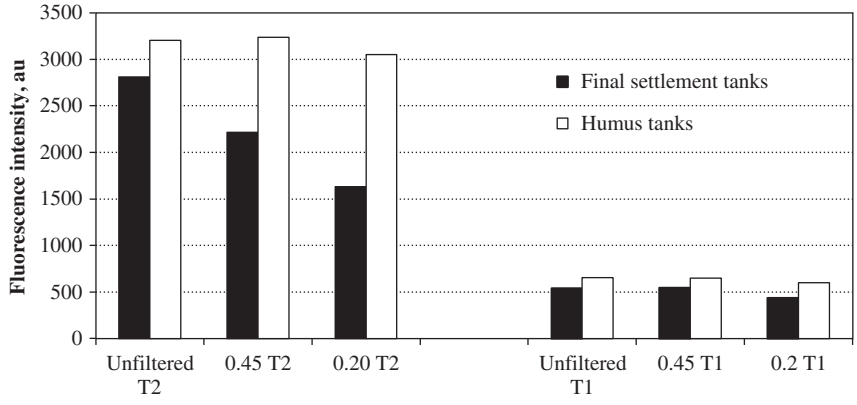

Figure 5. Mean Peak T1 and T2 values for unfiltered and filtered samples in FSTs and humus tanks over the sampling period.

and humus tanks. Standard deviations, maxima and minima data are provided in Table 2. It is clear that, whilst the humus tank values always exceed the FST values, there is little difference between the T1 values measured across the two streams. Of particular interest, however, is the observation that there are significant differences between filtered T2 intensities for the FSTs and humus tanks, indicating enhanced removal of the smallest fluorophores in the ASP. Furthermore, it is also apparent from Figure 5 that for both T1 and T2, the majority of the fluorescence is derived from the $<0.2\,\mu m$ fraction. Certainly, in the case of the humus tanks, there is little discernible difference between the fluorescence intensities of the bulk, 0.45 and $0.2\,\mu m$ fractions, indicating that fluorescence spectroscopy is monitoring

Table 2. Mean, standard deviation, maximum and minimum Peak T1 and T2 values for unfiltered and filtered samples in FSTs and humus tanks over the sampling period.

	Unfiltered T2	0.45 μm T2	0.20 μm T2	Unfiltered T1	0.45 μm T1	0.20 μm T1
Final settlement tanks						
Average	2811	2216	1631	540	544	436
SD	1848	1057	662	142	295	66
Max.	7324	46,427	2755	860	1243	550
Min.	1720	1197	465	385	315	357
Humus tanks						
Average	3203	3239	3051	656	650	602
SD	857	1320	1048	144	268	154
Max.	4477	5342	5395	855	1245	917
Min.	2058	1343	16,823	457	321	356

microbial exudates and <0.2 μm fractions of larger material which pass through the trickling filters and humus tanks, but are retained by the activated sludge reactor and final tanks.

Building on the work of Hudson et al. [10] and Reynolds and Ahmad [20] who identified correlations between trypotophan-like fluorescence and BOD_5 in surface waters, the data were similarly analysed. Relationships between the unfiltered and filtered samples and BOD_5 are shown in Figure 6, and correlation coefficient values (r) are given in Table 3.

It is apparent from Figure 6 that COD values are significantly greater than the corresponding BOD values. This is to be expected as the COD value is a measurement of the oxidation potential of a sample of (waste)water in terms of its biodegradable and non-biodegradable constituents, and so includes substances which are chemically oxidized as well as biologically oxidized, unlike BOD_5.

Very strong correlations can be seen in Table 3 between both BOD and fluorescence and between COD and fluorescence. In particular, considering the BOD_5 analyses, for each filter size (including unfiltered), the correlation between T1 and BOD_5 is always the strongest, and for two of the three relationships, T2 has the weakest correlation with BOD_5. For the COD analyses, T2 always exhibits the weakest relationship, and T1 exhibits the strongest for two of the three. Interestingly, it is the unfiltered relationship where T1 does not have the strongest correlation with COD; in this instance it is Peak C, although there is little absolute difference between the two values.

Comparing the correlations between BOD_5 and the different fluorescence peaks for each filter size with the corresponding COD correlations, it can be seen that the relationships between BOD and fluorescence peaks are stronger than those between COD and the same peaks, with the exception of Peak C, where the strength of correlation is the same for BOD_5 as it is for COD. For the 0.45 μm filtered samples, again BOD_5 correlations with fluorescence intensities are stronger than the COD correlations, except for Peak C. For the 0.2 μm samples, the two sets of correlations are similar in each case, again except for Peak C.

These data clearly demonstrate that there are relationships between water quality (or, more specifically, its biodegradable organic matter) and fluorescence in the Peak T fluorescence region. Interestingly, the results also demonstrate reasonable relationships between water quality and Peak C fluorescence, although relationships with Peak A are less well defined. Fluorescence within the Peak C region has been attributed to microbially and chemically reprocessed organic matter,[9] which would explain the strong correspondence between Peak C and Peak T fluorescence. The data also suggest that since the 0.2 μm samples exhibit such equally strong correlations as the unfiltered samples, and that the bulk of the total fluorescence is observed in the <0.2 μm fraction, then a significant proportion of fluorescence must be a consequence of this smallest fraction of organic matter present in the wastewater. Given the strong relationships between BOD and Peak T1 and COD and Peak T1, there is potential to use tryptophan fluorescence as a water quality indicator. In addition, using the same fluorescence techniques, Cumberland et al. [19] demonstrated reasonable correlations between Peak T2 and *E. coli* ($r = 0.87$) and between Peak T2 and HB ($r = 0.83$). Thus, there is the possibility for using fluorescence as an indicator of *E. coli* or HB removal, although more work is required to test these correlations on partially treated wastewater and final effluent.

These results represent a significant development, both in terms of the relationships between Peak T and BOD and COD, but also in identifying the changes in fluorescence with treatment, and the impact of different size fractions on fluorescence. The data presented here show that fluorescence spectroscopy has the potential to remove the industry's reliance on the outdated and inaccurate BOD test with an alternative assessment of microbiological quality, and incorporation into on-line, real-time measurement would facilitate a step change in wastewater quality assessment for the water industry. Murphy et al. [25] suggest that the deployment of a small (undefined) number of fluorometers at appropriate wavelengths would capture the same information as would be collected via on-line monitoring of complete EEMs. Furthermore, using the same instrument,

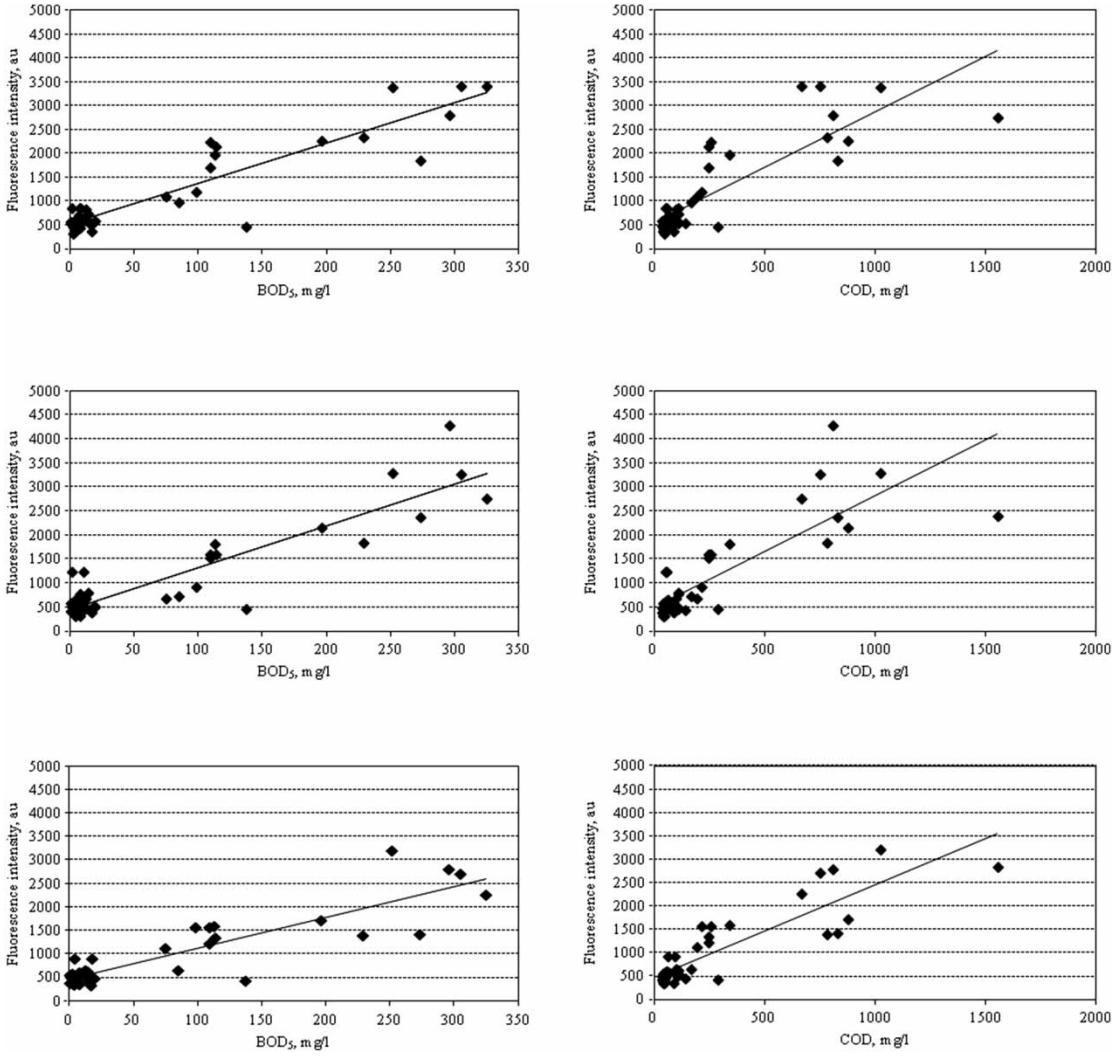

Figure 6. Relationship between Peak T1 and BOD_5, and between Peak T1 and COD for (a) unfiltered, (b) 0.45 μm and (c) 0.20 μm filtered samples.

Table 3. Relationship between fluorophores, BOD and COD at different filter sizes.

	BOD			COD		
	Unfiltered	0.45 μm	0.20 μm	Unfiltered	0.45 μm	0.20 μm
T1	0.923	0.909	0.891	0.852	0.835	0.881
T2	0.729	0.725	0.712	0.556	0.575	0.714
A	0.842	0.792	0.776	0.696	0.686	0.746
C	0.880	0.742	0.673	0.880	0.776	0.784

Cumberland et al. [19] found good correlation between Peak T1 fluorescence and the presence of *E. coli* and HB in water. It is possible, therefore, that in future, fluorescence could also be used as an indicator or *E. coli* removal in treatment processes. Further work is now required to develop further the relationships identified here and, with commercially available *in situ* probes now on the market, to consider the means by which such probes might be deployed successfully, so enabling the real-time, continuous, collection of data at WwTW.

Conclusions

(1) Fluorescence spectroscopy has been successfully applied to monitor wastewater treatment processes and to identify changes to wastewater character.
(2) The performance of WwTWs unit processes can be assessed through straightforward analysis of EEMs via peak-picking.

(3) Fluorescence data suggest that activated sludge plants are more effective than trickling filters and humus tanks at removal of the smallest ($<0.2\,\mu m$) organics fraction.
(4) Strong correlations exist between BOD_5 and Peaks T1, A and C in unfiltered samples, and between COD and Peaks T1 and C in unfiltered samples. Fractionation data demonstrate clearly that the majority of the fluorescence is derived from the $<0.2\,\mu m$ fraction.
(5) The results presented in this paper demonstrate the potential for fluorescence spectroscopy to be used as an alternative to BOD_5 in the operation and management of WwTWs. Work is now required to apply the technology to permit the real-time, continuous measurement of fluorescence to facilitate works optimization.

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References

[1] HMSO. 5-Day biochemical oxygen demand. 2nd ed. Methods for the examination of waters & associated materials. London: HMSO; 1988.
[2] Bourgeois W, Burgess JE, Stuetz RM. On-line monitoring of wastewater quality: a review. J Chem Technol Biotechnol. 2001;76(4):337–348.
[3] HMSO. Chemical oxygen demand (Dichromate Value of Polluted & Wastewaters. Methods for the examination of waters & associated materials. London: HMSO; 1977.
[4] HMSO. Chemical oxygen demand (Dichromate Value of Polluted & Wastewaters). 2nd ed. Methods for the examination of waters & associated materials. London: HMSO; 1986.
[5] Reynolds DM, Ahmad SR. Rapid and direct determination of wastewater BOD values using a fluorescence technique. Water Res. 1997;31(8):2012–2018.
[6] Hudson NJ, Baker A, Reynolds D. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters – a review. Rivers Res. 2007;23:631–649.
[7] Henderson RK, Baker A, Murphy KR, Hambly A, Stuetz RM, Khan SJ. Fluorescence as a potential monitoring tool for recycled water systems: a review. Water Res. 2009;43: 863–881.
[8] Bridgeman J, Bieroza M, Baker A. The application of fluorescence spectroscopy to organic matter characterisation in drinking water treatment. Rev Environ Sci Biotechnol. 2011;10:277–290.
[9] Ishii SKL, Boyer TH. Behaviour of reoccurring PARAFAC components in fluorescent dissolved organic matter in natural and engineered systems: a critical review. *Environ Sci Technol.* 2012;46:2006–2017.
[10] Hudson N, Baker A, Ward D, Reynolds DM, Carliell-Marquet C, Browning S. Fluorescence spectrometry as a surrogate for the BOD_5 test in water quality assessment: an example from South West England. Sci Total Environ. 2008;391(1):149–158.
[11] Bari A, Farooq S. Measurement of wastewater treatment efficiency by fluorescence and UV absorbance. Environ Monit Assess. 1985;5(4):423–434.
[12] Ahmad SR, Reynolds DM. Synchronous fluorescence spectroscopy of wastewater and some potential constituents. Water Res. 1995;29:1599–1602.
[13] Reynolds DM, Ahmad SR. The effect of metal ions on the fluorescence of wastewater wastewater. Water Res. 1995;29:2214–2216.
[14] Liu T, Chen Z-L, Yu W-Z, You S-J. Characterization of organic membrane foulants in a submerged membrane bioreactor with pre-ozonation using three-dimensional excitation–emission matrix fluorescence spectroscopy. Water Res. 2011;45:2111–2121.
[15] Hur J, Lee T-H, Lee B-M. Estimating the removal efficiency of refractory dissolved organic matter in wastewater treatment plants using a fluorescence technique. Environ Technol. 2011;32(16):1843–1850.
[16] Quaranta ML, Mendes MD, MacKay AA. Similarities in effluent organic matter characteristics from Connecticut wastewater treatment plants. Water Res. 2011;46: 284–294.
[17] Esparza-Soto M, Núñez-Hernández S, Fall C. Spectrometric characterization of effluent organic matter of a sequencing batch reactor operated at three sludge retention times. Water Res. 2011;45:6555–6563.
[18] Singh S, Henderson RK, Baker A, Stuetz RM, Khan SJ. Characterisation of reverse osmosis permeates from municipal recycled water systems using fluorescence spectroscopy: implications for integrity monitoring. J Memb Sci. 2012;421–422:180–189.
[19] Cumberland S, Bridgeman J, Baker A, Sterling M, Ward D. Fluorescence spectroscopy as a tool for determining microbial quality in potable water applications. Environ Sci Technol. 2012;33(6):687–693.
[20] Reynolds DM, Ahmad SR. Synchronous fluorescence spectroscopy of wastewater and some potential constituents. Water Res. 1995;29:1599–1602.
[21] Bieroza M, Baker A, Bridgeman J. Assessing organics removal in water treatment with data mining and artificial neural networks. Adv Eng Softw. 2012;44:126–135.
[22] Carstea EM, Baker A, Bieroza M, Reynolds D. Continuous fluorescence excitation–emission matrix monitoring of river organic matter. Water Res. 2010;44(18):5356–5366.
[23] Hambly A, Henderson R, Storey MV, Baker A, Stuetz RM, Khan SJ. Fluorescence monitoring at a recycled water treatment plant and associated dual-distribution system – implications for cross-connection detection. Water Res. 2010;44:5323–5333.
[24] Singh S, Henderson RK, Baker A, Stuetz RM, Khan SJ. Distinguishing stage 1 and 2 reverse osmosis permeates using fluorescence spectroscopy. Water Sci Technol. 2009;60:2017–2023.
[25] Murphy KR, Hambly A, Singh S, Henderson RK, Baker A, Stuetz R, Khan SJ. Organic matter fluorescence in municipal water recycling schemes: toward a unified PARAFAC model. Environ Sci Technol. 2011;45:2909–2916.