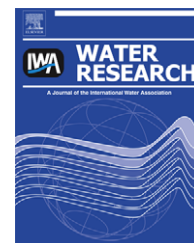


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Review

Fluorescence as a potential monitoring tool for recycled water systems: A review

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ABSTRACT

A rapid, highly sensitive and selective detector is urgently required to detect contamination events in recycled water systems – for example, cross-connection events in dual reticulation pipes that recycle advanced treated sewage effluent – as existing technologies, including total organic carbon and conductivity monitoring, cannot always provide the sensitivity required. Fluorescence spectroscopy has been suggested as a potential monitoring tool given its high sensitivity and selectivity. A review of recent literature demonstrates that by monitoring the fluorescence of dissolved organic matter (DOM), the ratios of humic-like (Peak C) and protein-like (Peak T) fluorescence peaks can be used to identify trace sewage contamination in river waters and estuaries, a situation analogous to contamination detection in recycled water systems. Additionally, strong correlations have been shown between Peak T and biochemical oxygen demand (BOD) in rivers, which is indicative of water impacted by microbial activity and therefore of sewage impacted systems. Hence, this review concludes that the sensitive detection of contamination events in recycled water systems may be achieved by monitoring Peak T and/or Peak C fluorescence. However, in such systems, effluent is treated to a high standard resulting in much lower DOM concentrations and the impact of these advanced treatment processes on Peaks T and C fluorescence is largely unknown and requires investigation. This review has highlighted that further work is also required to determine (a) the stability and distinctiveness of recycled water fluorescence in relation to the treatment processes utilised, (b) the impact of matrix effects, particularly the impact of oxidation, (c) calibration issues for online monitoring, and (d) the advanced data analytical techniques required, if any, to improve detection of contamination events.

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Nomenclature

BOD	biological oxygen demand	NF	nanofiltration
COD	chemical oxygen demand	NTU	nephelometric turbidity units
DO	dissolved oxygen	PARAFAC	parallel factor analysis
DOM	dissolved organic matter	PE	polyethylene
EEM	excitation–emission matrix	PLS	partial least squares
EPA	Environmental Protection Agency	PVC	polyvinyl chloride
EPS	extracellular polymeric substances	RO	reverse osmosis
EWS	early warning systems	SAT	soil-aquifer treatment
GRAMS	generalised rank annihilation models	SMP	soluble microbial products
IFE	inner filtering effect	SS	suspended solids
IPR	indirect potable reuse	THM	trihalomethane
MCR	multivariate curve resolution	TN	total nitrogen
MBR	membrane bioreactor	TOC	total organic carbon
MF	microfiltration	TP	total phosphorous
		UF	ultrafiltration
		WWTP	wastewater treatment plant

1. Introduction

Unpredictable rainfall patterns, in conjunction with population increases, are placing pressure on water resources in many urban areas. Consequently, water utilities around the world are developing action plans with the aim of ensuring water is managed sustainably while meeting customer demand. During the last two decades, reuse and recycling of treated sewage effluent have played an increasingly prominent role in achieving this goal (Miller, 2006; Anderson, 2007). For example, in a small number of new development areas in Australia and the US, treated sewage effluent is diverted back to residential estates for uses such as toilet flushing and garden irrigation by means of a dual reticulation system (de Rooy and Engelbrecht, 2003; Marks and Zadoroznyj, 2005; Anderson, 2007). Other schemes utilise highly treated municipal effluent that meets coliform, biochemical oxygen demand (BOD) and turbidity limits as determined by the appropriate authority to replenish potable water supplies, known as indirect potable reuse (IPR) (Drewes et al., 2003; Wintgens et al., 2008).

Dual reticulation and IPR schemes typically incorporate highly effective advanced treatment technologies including membrane filtration and disinfection processes (Wintgens et al., 2005). However, treatment failures and cross-connections between recycled water and potable water systems can occur. For example, a random audit of properties connected to a dual reticulation scheme in Sydney, Australia, identified 50 cross-connections prior to the deliverance of water to the system and at least four post-commissioning incidents have been reported since 2001 (de Rooy and Engelbrecht, 2003). Such incidents can seriously compromise both public health and customer confidence (Marks et al., 2003; Marks, 2007).

In water recycling schemes, there is a need for careful and on-going management to ensure reliability of water treatment performance to maintain full protection of public health. Cross-connections between potable water systems and recycled water pipes need to be rapidly identified. In order to ensure such reliability and to be able to take action quickly if treatment fails or if cross-connections are identified, some form of real-time water quality monitoring is urgently required. Current methods of online water treatment

monitoring systems are generally limited to a few simple parameters including conductivity, total organic carbon (TOC) and, in the instance of membrane processes, trans-membrane pressure. The development of monitoring systems suitable for the rapid assessment of process performance and water quality is a key area requiring further research (Wintgens et al., 2005; Foley et al., 2007).

Fluorescence spectroscopy has received attention in the water industry with respect to its potential application as a monitoring technique. Fluorescence monitoring is attractive as it is a rapid, reagentless technique that requires no sample preparation prior to analysis. Fluorescence spectroscopy has been investigated as a monitoring tool for a range of applications including water quality and pollution monitoring in rivers (Hudson et al., 2007), process control in sewage treatment works (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Hudson et al., 2007), specific pollutants in industrial wastewater (Kuzniz et al., 2007), oil in water (Lambert, 2003) and disinfection byproduct formation potentials in drinking water treatment (Hua et al., 2007b). We discuss the potential for utilising fluorescence as a monitoring tool for recycled water systems in this review. An evaluation of the advantages of using fluorescence over other commonly employed process monitoring options is provided. The challenges that will have to be overcome in order to implement the technology are considered.

2. Fluorescence versus other monitoring technologies

2.1. Established technologies: why investigate alternatives?

A wide range of chemical parameters are routinely measured for compliance and/or performance assessment of potable and wastewater. These include chlorine residuals, ozone, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), pH, conductivity, turbidity, colour, metals, dissolved oxygen, ammonia, nitrate and phosphate and this list is not exhaustive (Briggs and Grattan, 1990). TOC, conductivity and sulphate analyses have been identified as key parameters for assessing membrane integrity (Adham et al., 1998; Kumar et al., 2007). The most important parameters for cross-connection detection have not been researched extensively. However, Hall et al. (2007) examined the intrusion of non-chlorinated secondary effluent into a potable water system and determined that deviations from the baseline were observed for chloride, specific conductance, turbidity and TOC. Overall, these studies identify TOC, conductivity, pH and turbidity as the most appropriate existing technologies for monitoring cross-connection and membrane performance, and also suggest the potential for utilising chlorine measurements.

Data obtained via online monitoring of chemical parameters are affected by environmental, process and instrument related variability. For example, seasonal impacts such as increased rainfall and algal blooms can affect TOC, conductivity, pH and turbidity levels. The treatment processes operated will affect the resultant concentrations of each of

the aforementioned parameters. Finally, the reliability and sensitivity of the instrument used for measurement will determine the accuracy of the result. To illustrate, a literature survey of the variation in TOC concentrations of potable water and effluent (treated by advanced processes including membrane bioreactors (MBR), reverse osmosis (RO) and soil-aquifer treatment (SAT)) showed variations of between $0.4\text{--}6.6\text{ mgL}^{-1}$ and $0.1\text{--}6.7\text{ mgL}^{-1}$, respectively (Table 1). Conductivity was also shown to vary significantly between potable and advanced treated water at between $13\text{--}570\text{ }\mu\text{S cm}^{-1}$ and $65\text{--}1240\text{ }\mu\text{S cm}^{-1}$, respectively (Table 1). Variations between turbidity, chlorine and pH are generally much less as these parameters are more easily controlled at the process level. Overall, based on these reported values, it appears that TOC and conductivity are most suitable for detecting cross-connections between potable and recycled water supplies (Table 1). This conclusion is similar to that identified for monitoring membrane performance (Adham et al., 1998; Kumar et al., 2007). Gray (2003) also stated that online conductivity and TOC measurements are the most common monitoring technologies for recycled or reclaimed water systems.

However, TOC or conductivity for cross-connection monitoring of small intrusions less than 5% is only feasible if a difference of 3 mgL^{-1} or $700\text{ }\mu\text{S cm}^{-1}$ is maintained between potable and recycled water supplies. Table 1 demonstrates that this may not always be possible, particularly if RO is utilised. Given the trend toward implementing RO systems (Wintgens et al., 2005), attempting to observe cross-connections with either of these monitoring parameters will become more difficult as the TOC and conductivity values of potable and recycled water approach similar levels. In this instance, it would even be problematic to use these parameters to measure much larger intrusions.

A study investigating RO membrane integrity testing showed that online conductivity monitoring was less sensitive than TOC monitoring (Adham et al., 1998). A further study showed that conductivity was not a good predictor of viral rejection (Kitis et al., 2003). These studies indicate that targeting the organic component, as opposed to small ions via conductivity, is more indicative of membrane performance. The limitations associated with TOC for cross-connection detection highlight the need for additional technologies that offer greater sensitivity to changes in the water quality and character. It is noted, however, that the implementation of multivariate techniques for analysing combinations of these commonly used parameters could significantly improve the sensitivity of these parameters despite large variations, as described by Rosen and Lennox (2001).

2.2. The emergence of optical technologies: UV versus fluorescence

It has been proposed that improved technology for performance assessment of water recycling systems might include optical technologies (Foley et al., 2007). The application of optical technologies for monitoring wastewater treatment processes has been widely researched, particularly with respect to UV-vis absorbance spectroscopic techniques

Table 1 – Comparison of typical potable and recycled water parameters and the potential for using these parameters in detecting cross-connections.

Water quality parameter	Reported potable water values	Reported recycled water values	Accuracy (based on HACH online instruments)	Potential for use in cross-connection monitoring
TOC (mgL ⁻¹)	0.6–6.4 ^a	6.7 ^c	4%	Yes, >4% intrusion detectable for extreme values
	0.5–6.2 ^b	2.0–4.0 ^e		
	3.5 ^c	0.1–1 (RO) ^h		
	1.27–4.11 ^d	0.5–5 (MBR) ^h		
	0.4–2.8 ^e			
Conductivity (μS cm ⁻¹)	13–39 ^f	880–1000 ^g	0.2%	Yes, > 1% intrusion detectable for extreme values
	64–570 ^g	550–800 ⁱ		
		65–90 ^j		
		1084–1240 ^k		
Free chlorine (mgL ⁻¹)	0–0.98 ^f	0.05–0.5 ⁱ	5%	No
pH	7–9 ^b	<0.5 ^l	0.1%	No
	7.3–8.6 ^d	7.7 ⁱ		
Turbidity (NTU)	0.1–0.3 ^d	7.0 ^l	2%	No
		0.1–0.3 ⁱ		
		0.09 ^l		

a Duong et al. (2003).

b Karavoltzos et al. (2008).

c Unpublished data by authors.

d (Volk et al., 2002).

e Soil-aquifer treatment (SAT) (Drewes et al., 2003).

f Sydney Water Corporation (2007).

g (Toifl and O'Halloran, 2008).

h Reverse osmosis (RO) and membrane bioreactor (MBR) (Asano et al., 2007).

i Microfiltration (MF) and RO blend (Chapman, 2006).

j MF followed by RO (Watkinson et al., 2007).

k MF only (Al-Shammiri et al., 2005).

l Depth filtration, UV, Cl₂ (Sydney Water Corporation, 2004).

(Thomas et al., 1996; El Khorassani et al., 1999; Bourgeois et al., 2001; Langergraber et al., 2003; Le Bonte et al., 2003; Rieger et al., 2004; Thomas and Constant, 2004; Langergraber et al., 2006; Jeong et al., 2007) and, to a more limited degree, fluorescence spectroscopy (Ahmad and Reynolds, 1995; Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Reynolds, 2002; Vassel and Praet, 2002; Miyata and Nakahara, 2007).

In both UV–vis absorbance and fluorescence spectroscopy, absorbance of incident radiation causes the excitation of loosely held electrons within double and triple bonds. UV–vis absorbance is a measure of the absorbance of such radiation against wavelength according to the Beer–Lambert Law, $A = \log(P_0/P)$, where P_0 is the power of the incident radiation and P is the power after passing through a specific length of solution (Skoog et al., 2004). Absorption and excitation will be followed in some instances by relaxation of the electron to a stable configuration, stimulating the emission of a photon-termed fluorescence (Lakowicz, 1999). The specific excitation and emission wavelengths are characteristic of a particular molecular conformation – the fluorophore. At low concentrations, measured peak intensity is directly proportional to the concentration of the responsible fluorophore in solution. This relationship is obscured at higher sample concentrations as a result of absorption of excitation and emission light by the sample matrix, a phenomenon known as the “inner filtering effect” (Section 6.1). The precise spectral excitation/emission signature depends on the specific chemical structure

as well as other factors including sample matrix and temperature.

An important advantage of fluorescence is that sensitivity is typically 10–1000× that of UV absorption spectroscopy, with single-molecule detection being possible. This is because fluorescence is proportional to incident power intensity, whereas absorbance is proportional to the ratio of radiant powers (Skoog et al., 2004). A further advantage of fluorescence is the potential for discriminating between different sources of chromophoric DOM that absorb at similar wavelengths (McKnight et al., 2001; Stedmon and Markager, 2005a). Such advantages have enhanced the popularity of fluorescence versus UV–vis absorbance, particularly in the marine science field (Blough and Del Vecchio, 2002). Fluorescence has also been revealed to be the more sensitive tool when compared with UV–vis absorbance for tracing effluent organic matter in IPR systems (Drewes et al., 2003).

Despite recognition of the potential advantages of fluorescence over UV–vis absorbance monitoring, as well as demonstration of the viability of fluorescence monitoring in the wastewater treatment field, it is UV–vis absorbance spectroscopy that has been commercialised for wastewater monitoring (Langergraber et al., 2003; Van Den Broeke et al., 2006). However, logging and handheld fluorescence instruments that focus on chlorophyll fluorescence or highly fluorescent dyes emitting in the visible light region are used routinely for algal monitoring and dye tracing respectively. More recently, *in situ* instruments have been used in river

systems for monitoring dissolved organic character fluorescence at the shorter wavelength, UV light region (Spencer et al., 2007b). These examples demonstrate that the technology is available and waiting to be applied.

3. Fluorescence identification of sewage-derived dissolved organic matter

3.1. Fluorescence and EEM interpretation of sewage impacted water

Traditionally, fluorescence measurements have been presented as emission spectra following irradiation at a fixed excitation wavelength (Lakowicz, 1999). Further developments have allowed synchronous fluorescence scanning, which is the measurement of emission spectra at an offset of the emission wavelength minus the excitation wavelength ($\Delta\lambda$), with typical offsets between 20 and 60 nm (Miano and Senesi, 1992; Ahmad and Reynolds, 1995; Wu et al., 2006). Recent technological advances have allowed the rapid detection (<1 min) of 3-dimensional excitation–emission matrices (EEMs). Each EEM is a composite of emission scans from a single sample recorded at incrementing excitation wavelengths and arranged in a grid (excitation \times emission \times intensity) (Fig. 1). Hence, large amounts of data are collected from each sample, facilitating the application of a wide range of powerful statistical analyses. Such statistical analyses and comparison of the acquired data between studies can only be conducted with confidence after the spectra have been corrected to account for instrument specific wavelength responses resulting from differences between excitation light sources, monochromators and emission detectors (Coble et al., 1990). Compensation for attenuated fluorescence emission resulting from inner filtering must also be performed before statistical interpretations are applied (Section 6.1).

There are five key fluorescence peaks commonly observed in freshwater aquatic samples. These have been classified according to Coble (1996) as follows: Peak A – humic-like ($\lambda_{\text{ex/em}} = 237\text{--}260/400\text{--}500$ nm); Peak C – humic-like ($\lambda_{\text{ex/em}} = 300\text{--}370/400\text{--}500$ nm); Peak B – tyrosine-like ($\lambda_{\text{ex/em}} = 225\text{--}237/309\text{--}321$ nm and $\lambda_{\text{ex/em}} = 275/310$ nm); Peak T₁ – tryptophan-like ($\lambda_{\text{ex/em}} = 275/340$ nm); and Peak T₂ – tryptophan-like

($\lambda_{\text{ex/em}} = 225\text{--}237/340\text{--}381$ nm) (Hudson et al., 2007) (Fig. 1). The Peak C fluorescence at $\lambda_{\text{ex}} = 300\text{--}370$ nm has been further divided into two fluorescence centres in highly coloured, peaty waters (Baker, 2001; Baker et al., 2008). Specifically, fluorescence is reported to occur at $\lambda_{\text{ex/em}} = 320\text{--}340/410\text{--}430$ nm and at $\lambda_{\text{ex/em}} = 370\text{--}390/460\text{--}480$ nm. In this review, these peaks are referred to as Peaks C₁ and C₂, respectively. Stedmon and Markager (2005a) also report a peak at $\lambda_{\text{ex/em}} = 290\text{--}310/370\text{--}410$ nm in freshwater that had been encouraged to produce algae by manipulation of nutrient levels. This peak appeared similar to that previously reported by Coble (1996) as “Peak M – marine humic-like”, suggesting an association with biological activity.

EEMs acquired from sewage-derived dissolved organic matter (DOM) commonly exhibit additional horizontally oriented peaks at $\lambda_{\text{ex}} = 375$, 350 and 330 nm for λ_{em} of 410–450 nm (Fig. 1). These are characteristic of optical brighteners such as those used in washing powder (Hayashi et al., 2002; Takahashi and Kawamura, 2007; Hartel et al., 2008). Approximately 40–80% of optical brighteners are removed during wastewater treatment. However, while they are present in river water at low concentrations of approximately $0.5 \mu\text{g L}^{-1}$ (Poiger et al., 1998), they are not typically observed in the EEMs of sewage impacted rivers due to background fluorescence (Baker, 2001).

Sewage-derived material is rich in tryptophan-like (T₁ and T₂) fluorescence, and is observably different from clean reservoirs and rivers, where Peaks C₁, C₂ and A predominate (Fig. 1) (Hudson et al., 2007). This is because DOM originating from clean river water is dominated by natural organic matter derived from plant material whereas sewage-derived DOM is dominated by organic matter originating from microbial activity (Hudson et al., 2008). Such differences in spectral signatures have facilitated the tracking of sewage contamination in river waters (Galapate et al., 1998; Baker, 2001; Baker et al., 2003; Chen et al., 2003b; Baker et al., 2005; Holbrook et al., 2005; Hudson et al., 2008).

3.2. Correlations between fluorescence and other water quality parameters

The strong fluorescence signal of sewage-derived DOM has led to the investigation of fluorescence as an alternative to

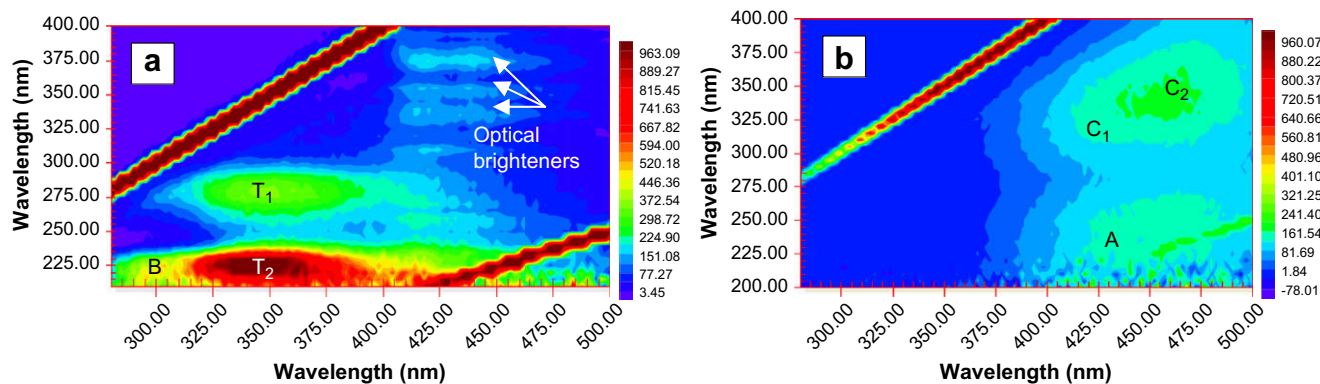


Fig. 1 – Fluorescence excitation–emission matrices (EEMs) for: (a) raw sewage and (b) clean river water, where B = tyrosine-like, T₁ and T₂ = tryptophan-like, A and C₁ = fulvic-like, C₂ = humic-like fluorescence.

existing water quality parameters commonly employed in the monitoring of river pollution (Baker, 2001; Baker and Inverarity, 2004; Hudson et al., 2008) and wastewater treatment processes (Bari and Farooq, 1985; Reynolds and Ahmad, 1997; Vassel and Praet, 2002; Miyata and Nakahara, 2007). Particular attention has been given to determine correlations between the fluorescence intensity of various peaks and other parameters including biochemical oxygen demand (BOD), total and dissolved chemical oxygen demand (COD), nitrate (NO_3^-), phosphate (PO_4^{3-}), total and dissolved organic carbon (TOC and DOC, respectively), $\text{UV}_{254, 340, 450}$, dissolved oxygen (DO) and ammonia (NH_3) (Table 2).

Correlations of Pearson's coefficient $r=0.77\text{--}0.98$ have been reported between BOD and Peaks T_1 and T_2 (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Baker, 2001; Ahmad et al., 2002; Baker and Inverarity, 2004; Hudson et al., 2008; Hur et al., 2008). In comparison, studies investigating the relationship between humic- and fulvic-like fluorescence (Peaks A and C_1, C_2) and BOD have found only moderate correlations ($r=0.72\text{--}0.77$) (Baker, 2001; Hudson et al., 2008), and in one instance no correlation was found (Comber et al., 1996). The stronger correlation between BOD and tryptophan-like fluorescence, as opposed to humic-like fluorescence, is expected because BOD is a measure of microbial growth, and it is tryptophan-like rather than humic-like fluorescence that has been associated with wastewater microbial activity (Reynolds, 2002; Elliott et al., 2006a; Hudson et al., 2008). A study of marine algae in experimental enclosures demonstrated increases in protein-derived fluorescence during the algal exponential growth phase, further evidencing the relationship between tryptophan-like fluorescence and microbial activity (Stedmon and Markager, 2005b). The same study found that humic-like fluorescence was produced during microbial degradation. Weak Peak T and BOD correlations, such as those found in river water where BOD values are small (Baker and Inverarity, 2004), probably reflect inaccuracies inherent in the BOD analytical technique when undertaken at BOD values close to the level of sensitivity (Comber et al., 1996).

The linking of fluorescence to other bulk organic parameters (COD, DOC and TOC) has been achieved with mixed success. Reported correlations for Peak T_1 and COD ranged from $r=0.42$ (or $r=0.56$ depending on the type of regression) (Vassel and Praet, 2002) and $r=0.51$ (Wu et al., 2006) to $r=0.96\text{--}0.97$ (Reynolds, 2002; Lee and Ahn, 2004). For Peak C_2 , a correlation of $r=0.87$ has been reported (Bari and Farooq, 1985). A similar range of correlations have been observed for relationships between fluorescence and TOC or DOC, with the exception of one sewage effluent study which revealed no correlation ($r=0.14$) between Peak C_1 and DOC (Cumberland and Baker, 2007). Such discrepancies can be explained by considering that DOC, COD and TOC parameters include both refractory and labile DOM of fluorescent and non-fluorescent character, and hence, correlations could be weakened by changes in the ratio of both humic/fulvic-like:tryptophan-like material and fluorescent:non-fluorescent DOM. For this reason, some investigators have found it useful to examine the $T_1:C_1$ ratio in relation to water quality. For example, Baker (2001) found that the $T_1:C_1$ for rivers was 1.0, far lower than that of untreated sewage at 2.7–31. Overall, for both BOD and COD measurements, closer correlations have been found

when sewage-derived fluorescence is more concentrated and thus dominates the system (Baker and Inverarity, 2004; Wu et al., 2006). For example, Wu et al. (2006) determined that while correlations between COD and Peak T_1 were weak ($r=0.5$) in samples taken on non-consecutive days, by obtaining samples on consecutive dry weather days when sewage-derived DOM was more concentrated, the correlation was improved to $r=0.9$.

Strong correlations have also been found between Peak T_1 and PO_4^{3-} , NO_3^- (Baker and Inverarity, 2004), Total Kjeldahl nitrogen (N_k) and NH_3 (Vassel and Praet, 2002). These relationships are generally considered to be indirect because sewage-derived pollution is typically characterised by high PO_4^{3-} and NO_3^- when advanced nutrient removal is not undertaken at the wastewater treatment plant (WWTP). Relationships between NH_3 and T_1 are not always observed because NH_3 is typically well removed during sewage treatment and only present in significant quantities during pollution events, as observed by Baker et al. (2003).

The examples shown in Table 2 demonstrate that, provided an appropriate sample set is evaluated, strong correlations between these traditional water quality parameters and fluorescence can be obtained. Recent publications (Hudson et al., 2007; Hudson et al., 2008) highlight that future research should focus on utilising and analysing fluorescence measurements as an independent test of water quality, rather than as a surrogate for well-known, traditional parameters that may be less meaningful.

4. Impact of treatment processes on fluorescence

An understanding of the impact of treatment processes on the fluorescence of the wastewater is required to determine the nature of the residual fluorescence signal, and thus the suitability of fluorescence as a monitoring tool in recycled water systems. A typical treatment process flow diagram for water reuse is provided in Fig. 2. Standard wastewater treatment generally comprises a series of sedimentation and biological processes designed to reduce suspended solids (SS), BOD, COD and total nitrogen, with phosphorus removal achieved by biological or chemicals means (Stages A–D). In the US, BOD and SS effluent limits are both 30 mgL^{-1} (Tchobanoglous et al., 2003). However, when treating water to an advanced standard for use in dual reticulation systems or indirect potable reuse, far more stringent requirements are required, for example 5 mgL^{-1} BOD, an average turbidity of 0.1 NTU, 1 mgL^{-1} TOC and $1/100\text{ mL}^{-1}$ coliforms (Environmental Protection Agency, 2004). These low levels can be achieved by including advanced treatment technologies, for example membrane treatment preceded by upstream filtration, followed by chemical or photolytic disinfection (Stages E–H).

The fluorescence character of water treated by processes A–D (Fig. 3) has been investigated using traditional 2-dimensional fluorescence (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Reynolds, 2002). A reduction in BOD throughout the process train (Fig. 3) and, in accordance with BOD– T_1 correlations (Table 1), a corresponding decrease in tryptophan-like fluorescence is observed. It has been

Table 2 – Summary of correlations found between fluorescence peak intensities of sewage impacted water and common water monitoring parameters.

System	Instrument	Peaks reported	Parameters measured	Correlations reported (peak/parameter/ Pearson's r unless marked)		Reference	
River water (62 sites within catchment)	Perkin–Elmer LS-50B luminescence spectrophotometer	A	PO ₄ ³⁻	T ₁	PO ₄ ³⁻	0.8	Baker and Inverarity (2004)
		C ₁	NO ₃ ⁻		NO ₃ ⁻	0.87	
		T ₁	BOD				
		T ₂	DO	T ₂	BOD	0.85	
		B	NH ₃		NH ₃	0.7	
			UV _{254, 340, 410}		DO	-0.65	
River water (12 sites within catchment)	Perkin–Elmer LS-50B luminescence spectrophotometer	T ₁	Conductivity	C ₁	TOC	0.68	Baker (2002b)
		C ₁	TOC				
		C ₂	UV _{254, 340, 410}				
Effluent (sewage and trade including pollution incidents –223 samples) and surface water (246 samples)	Varian Cary Eclipse fluorescence spectrophotometer	T ₁	BOD ₅	For the entire data set:			Hudson et al. (2008)
		T ₂	TOC	T ₁	BOD ₅	0.906 ^a	
		C ₂			TOC	0.876 ^a	
		A		T ₂	BOD ₅	0.848 ^a	
					TOC	0.802 ^a	
				C ₂	BOD ₅	0.771 ^a	
					TOC	0.87 ^a	
		A	BOD ₅	0.72 ^a			
			TOC	0.808 ^a			
Treated effluent (4 collected over 3 month period)	Perkin–Elmer LS-50B luminescence spectrophotometer	T ₁	COD	T ₁	COD	0.96 ^c	Lee and Ahn (2004)
		T ₂	SS				
		C ₁					
		A					
Sewage effluent (16 samples)	Varian Cary Eclipse fluorescence spectrophotometer	C ₁	DOC	C ₁	DOC	0.14	Cumberland and Baker (2007)
		C ₂					
Sewage in rivers (10 sites at 6 rivers)	Perkin–Elmer LS-50B luminescence spectrophotometer	C ₁	UV ₂₅₄	T ₁	UV ₂₅₄	0.85	Baker (2001)
		T ₁	Cl ⁻		BOD ₅	0.77	
			SS, BOD				
			NH ₃	C ₁	UV ₂₅₄	0.81	
			DO, pH	BOD ₅	0.72		
Urban river, WWTP effluent, reservoir discharge – 18 samples	Perkin–Elmer LS-55 luminescence spectrophotometer	T ₁	Conductivity	T1	BOD	0.9	Hur et al. (2008)
		C ₁	DO, SS				
			BOD				
			TN, NH ₃				
			NO ₃ ⁻				
			TP, PO ₄ ³⁻				
			Cl ⁻ , DOC				

(continued on next page)

Table 2 (continued)

System	Instrument	Peaks reported	Parameters measured	Correlations reported (peak/parameter/Pearson's r unless marked)			Reference
Sewage (treated and untreated sewage from one WWTP)	Perkin-Elmer LS-50B luminescence spectrophotometer	T ₁	BOD COD TOC	T ₁	BOD COD TOC	0.96 0.97 0.96	Reynolds (2002)
Raw sewage (70 samples) and treated effluent (59 samples)	Fluoromax™, SPEX instruments, SA	T ₁ C ₁	BOD	T ₁	BOD	0.96 _(STW A) 0.97 _(STW B) 0.94 _(STW C)	Reynolds and Ahmad (1997)
Treated sewage effluent	KrF excimer laser and Cassegrain telescope	T ₁ -T ₂	BOD	T ₁ -T ₂	BOD	0.98	Ahmad and Reynolds (1999)
Filtered raw sewage	Perkin-Elmer LS-50B luminescence spectrophotometer	T ₁	COD TOC N _k NH ₄ -N Conductivity	T ₁	COD TOC N _k NH ₄ -N	0.42 0.41 0.69 0.65 0.56 ^b 0.53 ^b 0.76 ^b 0.87 ^b	Vasel and Praet (2002)
Ferrate treatment of effluent, raw sewage and mixtures of the two (23 samples)	Turner Fluorometer model 111	C ₂	COD	C ₂	COD	0.87	Bari and Farooq (1985)
Sewage effluents (10)	Perkin-LS5 fluorometer	C ₁ A	BOD	C ₁ A	BOD BOD	No correlation No correlation	Comber et al. (1996)
Wastewater (96 samples taken over 4 non-consecutive days)	Jobin-Yvon JY3 fluorometer or Perkin-Elmer LS-50B luminescence spectrophotometer	T ₁ C ₁	DCOD _(dissolved) COD _{total} NH ₃	T ₁	DCOD COD	0.37 0.51	Wu et al. (2006)
Two sewage pollution events (19 samples)	Perkin-Elmer LS-50B luminescence spectrophotometer	T ₁ C ₁	NH ₃ TOC UV ₂₅₄ Conductivity	T ₁	NH ₃	0.96	Baker et al. (2003)

a Spearman's ρ .b With partial least squares (PLS) analysis using $n = 30$.

c After correction for dissolved solids.

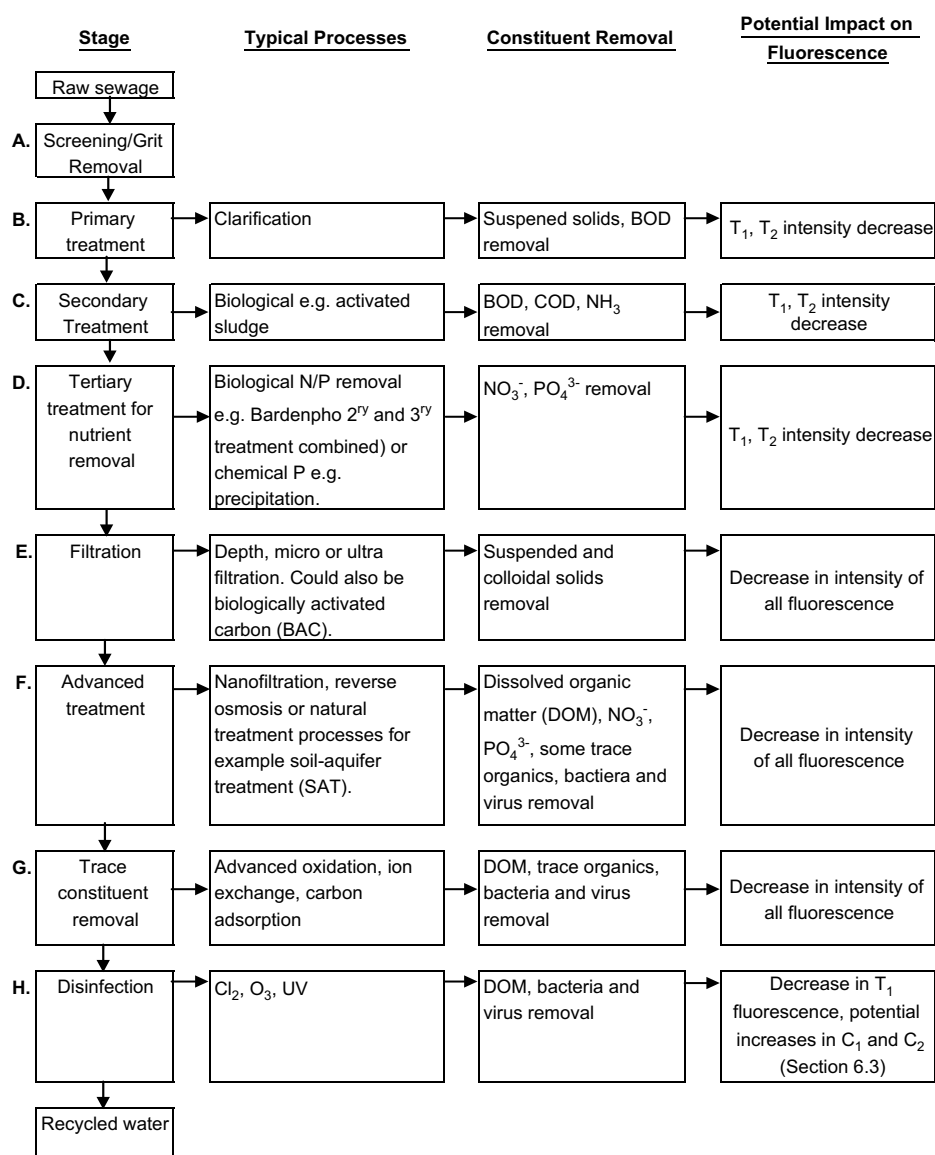


Fig. 2 – Typical treatment processes A–H utilised in the production of recycled water.

proposed that sewage effluent fluorescence characteristics are typical of those exhibited by soluble microbial products (SMP) and extracellular polymeric substances (EPS) and as such comprise Peaks T₁, T₂ and C₁, but not C₂ (Esparza-Soto and Westerhoff, 2001; Westerhoff et al., 2001; Chen et al., 2003b; Her et al., 2003; Lee and Ahn, 2004; Sheng and Yu, 2006). This is supported by a study that characterised both wastewater effluent and water not impacted by anthropogenic sources using XAD resin fractionation where it was found that wastewater effluent, in contrast to natural water, contained fulvic and hydrophilic acids, but minimal humic acid (Ma et al., 2001). Furthermore, a recent study in the marine environment identified CDOM components at Peak C₁ that originated from both terrestrial and marine environments while finding that Peak C₂ fluorescence was only derived from terrestrial sources (Murphy et al., 2008). Interestingly, when investigating COD removal by biological processes, Lee and Ahn (2004) observed that none of the refractory fulvic-like

peak was removed by wastewater treatment. It may therefore be expected that the T₁:C₁ ratio would decrease after wastewater treatment, although this supposition requires verification.

Relatively little research has been conducted on the fluorescence of wastewater subjected to advanced treatment technologies (Processes E–G, Fig. 2). The literature examined suggests that reducing BOD values to <10 mgL⁻¹ will cause a decrease in tryptophan-like fluorescence (Table 2). A recent review of the treatment of effluent organic matter determined that DOC removal by UF, NF, MBR and RO membrane processes was in the range 10–58%, 40–92%, 88–98% and 85–99%, respectively (Wintgens et al., 2005; Shon et al., 2006). Hydrophilic, neutral compounds are most likely to remain at trace levels in the membrane permeate while hydrophobic, charged DOC is rejected (Drewes et al., 2003; Hu et al., 2003; Snyder et al., 2007). Hence, fulvic-like material – a hydrophobic, charged fraction – is likely to be removed, implying

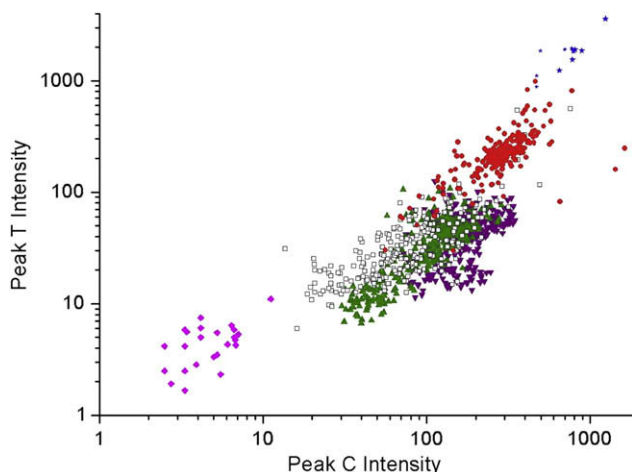


Fig. 3 – Blue stars: untreated wastewaters (mains sewer or STW influent); Baker (2005). Red circles: STW final treated effluent (Hudson et al., 2008; Baker, 2005). Black squares: river water, SW England (Hudson et al., 2008). Purple down triangle: river water, C England (Bierozza et al., in press). Green up triangles: clarified drinking water (Bierozza et al., in press). Magenta diamonds: laboratory deionised water, 18M-ohm (unpublished data). All data collected from one instrument and normalised to a constant raman intensity. Data from Baker (2005) dissolved organic matter analysed at 10 °C; all other data unfiltered organic matter analysed at 20 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that C_1 fluorescence intensity will be reduced by advanced treatment.

Finally, it is known that oxidants, for example ozone and chlorine, can increase or decrease fluorescence intensity (Korshin et al., 1999). Tryptophan-like fluorescence in particular has been observed to decrease (Świetlik and Sikorska, 2004) (Section 6.3). One study investigating the fluorescence signature of reclaimed water treated by nitrification/ denitrification, disinfection and tertiary filtration revealed that strong tryptophan-like peaks persisted following treatment. Upon subsequent soil-aquifer treatment the tryptophan-like fluorescence signal was reduced but still observable and contrasted with the fluorescence signal of a drinking water sample which was dominated by humic-like fluorescence (Drewes et al., 2006).

5. Data handling and visualisation

The number, composition and structure of fluorophores in natural organic matter samples are variable and essentially unknown. Overall, researchers have tended to favour simple techniques for summarising EEMs, typically utilising visual identification of fluorescence peaks, as illustrated in Fig. 1. It has been found that the intensities of some peaks and their ratios can be useful for monitoring and discriminating between organic matter sources in soil (Antizar-Ladislao et al., 2006),

ivers (Baker and Spencer, 2004; Liu et al., 2007), lakes (McKnight et al., 2001), oceans (Coble et al., 1990; Coble et al., 1998), effluents (Baker, 2002a; Saadi et al., 2006) and ships' ballast water (Murphy et al., 2004). However, such techniques make only minimal use of the large quantity of data represented in each EEM. Chen et al. (2003b) divided wastewater EEMs into five operationally defined sections associated with peaks of different compound types and integrated and normalised the data within these boundaries. While increasing the quantity of data contributing to the EEM characterisation, other characteristics of potential interest such as peak location which may shift due to the presence of overlapping spectra or chemical interferences, are not retained using this method.

In recent years, there has been shift toward greater sophistication in interpreting fluorescence EEMs via the application of multivariate analysis and multiway techniques that leverage the information inherent within data of higher-order structure (Bro, 1997; Escandar et al., 2007). Chemometric techniques that have been used to parameterise wastewater EEMs include principal components analysis (PCA) (Tartakovsky et al., 1996), partial least squares regression (Vassel and Praet, 2002) and principal filter analysis (Brunsdon and Baker, 2002). Parallel factor analysis (PARAFAC) has been used extensively by analytical chemists analysing fluorescence EEMs (for reviews see Bro (1997) and Bro (2006)) but was not applied to natural organic matter fluorescence until recently (Stedmon et al., 2003). PARAFAC utilises the trilinear structure of an EEM dataset to estimate the concentrations and spectra of underlying fluorescent components, and can reveal true spectra even in the presence of uncalibrated spectral interferences (the "second-order advantage") (Bro, 1997; Murphy et al., 2006). Other multiway models such as generalised rank annihilation models (GRAMS), Tucker models, multiway-PLS and multivariate curve resolution (MCR) can also yield useful information from fluorescence EEMs (Comas et al., 2004; Bro, 2006).

Multiway analysis is slowly gaining traction in the analysis of wastewater DOM. In a study of a Melbourne WWTP, PCA and PARAFAC were used to distinguish between changes in water quality over time attributable to nitrification and denitrification, seasonal variability and changes in plant process conditions (Miettinen et al., 2004). Bergamashi et al. (2005) used PCA to characterise the contribution of EEM fluorescence endmembers for various agricultural and sewage treatment sources within a watershed. Several authors have used PARAFAC to classify EEMs according to broad water sources including wastewater (Hua et al., 2007a; Wang et al., 2007). However, there are as yet no detailed studies of wastewater or recycled water treatment systems that relate fluorescence measurements to process failures or membrane under-performance. It is possible that by utilising techniques such as PARAFAC in the analysis of wastewater, interfering fluorescence originating from synthetic organic compounds could be distinguished and removed mathematically. For example, the fluorescence peaks of optical brighteners, if present, can overlap with fluorescence from humic-like materials. By distinguishing the independently varying natural and synthetic fluorescent components, PARAFAC analysis could facilitate the quantitative analysis of both humic-like substances and residual optical brighteners.

The development of online monitoring devices capable of applying predictive algorithms in real time will test the ultimate utility of multivariate approaches for monitoring WWTP performance (Tartakovsky et al., 1996; Langergraber et al., 2003; Amigo et al., 2008). While significant strides have been made in this direction, there are several challenges to use multivariate statistics that must be overcome, including the screening of unreliable data, the handling of variables that are collinearly or non-linearly related, and the detection of deviations against cyclical or non-stationary process conditions (Rosen et al., 2003).

6. Challenges to implementation: matrix effects

Within the field of analytical chemistry, “matrix effects” refer to the combined effect upon the sample measurement of the components in a sample other than the analyte. These effects may be caused by chemical or physical means. Matrix effects introduce a significant challenge to performance monitoring by interfering with either the detection or behaviour of the variables of interest.

6.1. Inner filtering effect

Within the context of fluorescence measurement, the inner filtering effect (IFE) refers to an apparent decrease in emission quantum yield and/or a distortion of bandshape as a result of the absorption of excited and emitted radiation by the sample matrix. In most natural and engineered systems, it is naturally dissolved humic material that has been shown to be highly susceptible to inner filter effects (Mobed et al., 1996; Ohno, 2002). Various authors have suggested different approaches to compensate for IFE, the two most common being an empirical correction based on the absorbance profile of the same sample (Parker and Barnes, 1957; Lakowicz, 1999) and simple sample dilution to a concentration at which IFE effects are negligible (Baker and Curry, 2004; Baker et al., 2004). An alternative approach is to leave the data uncorrected, and utilise the resulting wavelength-dependent non-linear relationship between fluorescence intensity and concentration.

All approaches have advantages and disadvantages when it comes to analysis of recycled water. Correction based on the absorbance profile increases analytical error through additional measurement. Dilution of concentrated solutions has a practical simplicity and does not require the determination of absorbance; however, the correction dilution or dilution range needs to be determined. Previous studies have used a range of dilutions varying from $\times 1$ to $\times 10$ for treated sewage effluents, $\times 10$ to $\times 100$ for untreated sewage (Baker et al., 2004) and over $\times 100$ for some landfill leachates (Baker and Curry, 2004). At very high dilutions, the fluorescence signature of any dilution water has to be carefully considered as a possible interferent. Retaining data in an uncorrected state may be most appropriate where only a simple regression against concentration is required rather than information on molecular character or structure. In these cases, the concern is at very high concentrations where a decrease in concentration may lead to an increase in fluorescence intensity.

Alternatively, absorbance is often used as a tool for determining if correction is needed. For example, when using a 10 cm cuvette at 300 nm, an absorbance of less than 0.02 ensured no inner filtering (Green and Blough, 1994), while for a 1 cm cell, IFE is considered negligible for samples exhibiting absorption at or below 0.05 across the wavelength range of measurement (Kubista et al., 1994). The threshold absorbance value will vary depending on absorbance method and chromophoric organic matter present. Hudson et al. (2008) demonstrated that, for river and treated wastewaters, inner filtering is unlikely to occur in samples with a TOC of <25 ppm.

6.2. Fluorescence quenching

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a molecule. A variety of processes can cause fluorescence quenching, such as excited state reactions, energy transfer, complex formation and molecular collision (Lakowicz, 1999). This phenomenon may pose challenges for the implementation of fluorescence spectroscopy for water quality monitoring method due to the complex and variable water matrices that exists within water recycling systems and distribution networks. The potential influence on fluorescence measurements from temperature variations, pH, metal ions and oxidation processes need to be considered.

6.2.1. Temperature

Fluorescence intensity is highly dependent on temperature. A rise in temperature increases the likelihood that an excited electron will return to its ground state by radiationless decay, leading to reduced fluorescence intensity. For example, fluorescence intensity can increase by $\sim 1\%$ with a 1°C decrease in temperature, within the range $10\text{--}45^\circ\text{C}$, for tryptophan-like, humic-like and fulvic-like substances depending on colloid size and fluorophore (Vodacek and Philpot, 1987; Baker, 2005; Elliott et al., 2006b; Sereďyńska-Sobecka et al., 2007). Within the context of recycled water schemes, water temperature may vary across a range of 20°C or more between summer and winter, leading to a corresponding minimum in fluorescence intensity during the summer by 20%. However, no research has yet been undertaken on the thermal quenching properties of recycled waters. Once determined, thermal quenching effects should be overcome by applying simple correction factors, as the effect of temperature on fluorescence quenching is linear and reversible (Vodacek and Philpot, 1987), or by the measurement of samples at a constant temperature. It is of note that different fluorophores of different size fractions may have different correction factors (Sereďyńska-Sobecka et al., 2007).

6.2.2. pH

A number of studies have shown that water pH can affect the fluorescence intensity and spectral shapes of humic- and fulvic-like substances and to a lesser extent, tryptophan-like substances (Vodacek and Philpot, 1987; Patel-Sorrentino et al., 2002; Baker et al., 2007; Spencer et al., 2007a). For example, Peak A relative fluorescence intensity (RFI) was observed to increase with pH 2–10 then decrease slightly between pH 10 and 11 while Peak C₁ RFI increased for pH 2–4 and stabilised between pH 4

and 10 before decreasing (Patel-Sorrentino et al., 2002). Spencer et al. (2007a) observed that Peak C_1 increased markedly between pH 2 and 6 and decreased at higher pH 8–10. In contrast, no decrease was reported for Peak C_2 at higher pH values and no significant pH effect was reported for Peak T_1 . The same study also noted a distinct red shift for Peak C_2 with increasing pH. Additionally, pH affects the solubility of metal ions which can impact on the fluorescence (Section 6.2.3).

Three mechanisms have been discussed to account for the observed pH effect as reviewed by Patel-Sorrentino et al. (2002): (a) the alteration of the molecular orbits of excitable electrons, (b) physical changes to molecular shape caused by changes in charge density and (c) competition between metal ions and H^+ . Studies have demonstrated that such changes are reversible from pH 2 to 12 indicating that major structural changes are not occurring (Vodacek and Philpot, 1987; Patel-Sorrentino et al., 2002).

However, most early studies focussed on model or extracted organic standards that were in the dissolved form, and significant pH effects on fluorescence intensity and wavelength were observed, for example, Mobed et al. (1996). Baker et al. (2007) found that in the analysis of both colloidal and dissolved samples of river water, pH effects were less important than size fraction variability in fluorescence for some fluorophores, particularly that of tryptophan-like fluorescence which was more commonly observed in colloidal fractions. For all fluorophores, the effect of pH was more variable than under controlled laboratory conditions.

6.2.3. Metal ions

Metal ions can originate in recycled water via three major routes. Influent raw sewage can contain high concentrations of heavy metals including chromium, copper, cadmium, lead, mercury, nickel, zinc, vanadium, iron and aluminium predominantly due to industrial effluents (Ahlwalia and Goyal, 2007). Chemical treatment processes such as coagulation introduce iron, aluminium and sodium to the system. Finally, leaching of metals from pipework in the distribution system can occur. For example, dissolved copper has been shown to leach into potable water systems by corrosion of copper piping (Jones-Lepp et al., 2001) while new PVC piping has been shown to leach lead (Wong et al., 1988) and organotin substances (Forsyth and Jay, 1997; Jones-Lepp et al., 2001; Impellitteri et al., 2007).

It has been known for many years that paramagnetic metal ions can quench humic-like fluorescence (Banerjee and Mukherjee, 1972; Levesque, 1972) via complexation reactions (Frimmel and Hopp, 1986). Humic-like quenching has been reported by Cu^{2+} , Fe^{3+} , and Hg^{2+} (Antunes et al., 2007), polyferric sulphate (Cheng and Chi, 2002), Ni^{2+} and Zn^{2+} (Provenzano et al., 2004). Fulvic-like quenching has been reported by Cu^{2+} and Pd^{2+} at pH 4 and 5 (Elkins and Nelson, 2002), Cu^{2+} at pH 6 (Luster et al., 1996; Esteves Da Silva et al., 1998) and Fe^{3+} at pH 4 (Esteves Da Silva et al., 1998). In contrast, diamagnetic ions such as Al^{3+} , Mg^{2+} , Ca^{2+} and Cd^{2+} can quench, enhance or have little effect on fluorescence (Elkins and Nelson, 2002). For example, on addition of Al^{3+} at pH 4 the relative fluorescence intensity (RFI) of fulvic acid was enhanced (Elkins and Nelson, 2002) while a different study recorded maximum fluorescence quenching of humic

substances at pH 5–5.5 (Vodacek and Philpot, 1987). Another study observed enhancement of fulvic acid fluorescence at pH 5 but quenching at pH 7.5 (Cabaniss, 1992). Sharpless and McGown (1999) observed both enhancement and quenching of humic acid fluorescence by Al^{3+} and demonstrated that precipitation accounted for the observed quenching.

Quenching of tryptophan-like fluorescence has been reported by Cu^{2+} and Ni^{2+} (Tabak et al., 1989), by copper, iron, nickel and aluminium for settled sewage samples (oxidation state not stated) (Reynolds and Ahmad, 1995), Cu^{2+} at pH 6 (Luster et al., 1996) and by Cu^{2+} , Fe^{3+} and Mo^{3+} (Tao et al., 1994). Additionally, the latter study demonstrated that Zn^{3+} , Mn^{2+} , Cr^{3+} , Co^{2+} , Ca^{2+} and Na^+ did not exhibit quenching effects. Overall, the degree of quenching varies depending on the fluorophore, metal ion concentration relative to the fluorophore concentration, metal ion speciation and pH conditions.

While there is evidence for metal-ion quenching of natural dissolved organic matter, almost all the published literatures involved laboratory based studies and the addition of free metal ions. In natural and engineered environments, metals (and organics derived from pipework) are most often in a particulate or organo-colloidal form, whose nature varies with pH (Lead and Wilkinson, 2006). Hence, the actual extent of fluorescence quenching in environments relevant to recycled water is largely unknown. Published results from natural and engineered systems do not show a clear metal ion quenching effect. Studies have suggested that metal quenching effects of tertiary treated wastewater and, by inference, recycled water could be minimal. For example, an investigation on the effect of metal ions on wastewater fluorescence demonstrated that for samples of final effluent, iron, aluminium and nickel metal ions appeared to have no impact on fluorescence intensity (Reynolds and Ahmad, 1995). Correlations presented in Table 2 further confirm that the presence of metal ions do not have a significant impact on fluorescence.

6.3. Oxidants

Oxidation processes, including chlorination, ozonation and advanced oxidation processes are frequently utilised in the provision of recycled water (Fig. 2). The impact of oxidants on recycled water fluorescence therefore must be considered.

The impact of chlorination on humic substance fluorescence was investigated by Korshin et al. (1999) who determined that for Cl_2/DOC ratios of <2 and >2 the fluorescence intensity of Peak C_1 increased and decreased, respectively. A later study reported that, for a Cl_2/DOC ratio of 0.8, the intensity of fluorescence exceeded that of the untreated samples at all times; however, at increased ratios the intensity of Peak C_1 decreased at reaction times greater than 10 min (Fabbicino and Korshin, 2004). Beggs et al. (2006) also determined that NOM reacted with chlorine over time (2 h – 5 days) causing a decrease in fluorescence intensity. In all cases chlorination caused a shift to shorter emission wavelengths which increased with higher chlorine doses and reaction times. Such observations have been explained by the fragmentation of humic substance since previous research has shown that fluorescence enhancement can be associated with

a decrease in average molecular weight (Peuravuori et al., 2002; Chen et al., 2003a). A study on the impact of ClO_2 and O_3 on XAD resin fractionated NOM determined that the wavelength shift and intensity increases were oxidant and fraction dependent (Świetlik and Sikorska, 2004). Interestingly, humic substances either increased in intensity or remained stable, while for protein peaks, a decrease in intensity was typical.

Advanced oxidation typically causes a decrease in overall fluorescence intensity. For example, Hofbauer and Andrews (2004) saw a decrease in fluorescence intensity of humic material with application of both UV alone and UV/hydrogen peroxide, although this was more prevalent for the latter. This was attributed to the decrease in molecular weight where it was postulated that hydroxyl radicals were attacking high electron density regions within double bonds. Similarly, Uyguner et al. (2007) determined when using synchronous scan that a 50% decrease in fluorescence intensity was observed upon ozonation of humic acid which increased to 70% upon sequential oxidation (ozonation followed by photocatalysis). In contrast, photocatalysis alone led to selective fluorescence enhancement of certain peaks, reinforcing the complexity of the process. It can therefore be hypothesised that while oxidation alone fractionates the chromophoric DOM into smaller chromophoric fractions, enhancing fluorescence, advanced oxidation succeeds in breaking chromophoric groups within the structure, decreasing fluorescence.

The effect of oxidants on fluorescent components within DOM is complex and as such it is difficult to ascertain the precise impact on recycled water fluorescence. Based on the literature, it can be proposed that under high chlorine doses, for example during hyperchlorination with long contact times, an overall decrease in fluorescence intensity may be observed, possibly affecting tryptophan-like fluorescence more than other fluorophores. Further work is required to assess the precise impact of oxidants on recycled water fluorescence.

7. Overall implications for fluorescence monitoring

The success of fluorescence as a recycled water monitoring tool for process performance assessment, cross-connection detection and water quality in IPR systems will lie in its ability to identify very small changes in DOM concentration and characteristics. The high sensitivity of fluorescence suggests that it would be suited to detect small changes in TOC concentration, providing that the TOC is at least partially fluorescent. Furthermore, it has been demonstrated that through the use of fluorescence EEM spectroscopy, characterisation of the fluorescent DOC can be achieved. For example, it is possible to differentiate between refractory organics such as fulvic and humic acids (Peaks A and C) and labile organics such as those associated with microbial activity (Peaks T and B). Given that refractory organics predominate in natural water while labile organics predominate in wastewater (Fig. 1), there is a potential to differentiate between drinking water and recycled water even if TOC concentrations are similar. Further comparisons of the literature have confirmed this as various waters of differing organic characters are separated by an order of magnitude on a log-log plot of Peak T versus Peak C

intensities (Fig. 3). Fig. 3 demonstrates that focussing in the fluorescent regions of either Peak T or Peak C would allow distinction between untreated wastewater, treated wastewater and river or drinking water. Such an approach has already been shown to be successful where an analysis of fluorescence peak ratios enabled the identification of highly treated reclaimed water in the receiving streams/groundwater of IPR systems (Holbrook et al., 2005; Drewes et al., 2006). These preliminary studies are encouraging although it is important to consider that the fluorescence spectra of advanced treated effluent will vary significantly as a result of a wide range of process trains being utilised to produce the quality required by the end use. Hence, further work is required to elucidate the applicability of fluorescence techniques to a wide variety of recycled water systems.

Efficient, automated data analysis is a key to implementing fluorescence as a monitoring technique. While some success has been achieved with simple peak picking (Holbrook et al., 2005; Drewes et al., 2006); the potential for greater sensitivity and selectivity using multivariate and multiway (i.e. three dimensions or higher) data analysis requires elucidation. Multiway tools such as PARAFAC have an unexplored potential for tracking cross-connections of drinking water and recycled water in a dual reticulation system, through revealing low-level underlying fluorescent components that might distinguish between water sources. Further work is required to determine which data analysis techniques are most useful in monitoring recycled water systems. If advanced analytical techniques are required, one challenge will lie in automation such that online monitoring and real-time decision-making are possible.

It has been shown that matrix and quenching effects can both affect the fluorescence of organic matter. Table 3 summarises the relevance of each of the discussed matrix effects with respect to implementation of fluorescence as a monitoring tool. Importantly, the anticipated impacts of inner filtering, temperature, pH and metal ions are shown to be minor or at least controllable. A more major impact is anticipated for the complex matrix effects of oxidants and further investigation is required in this regard. However, the majority of published research on this topic involves laboratory studies on the behaviour of dissolved organic matter standards in simple controlled experiments. Further work is required to elucidate the impact of oxidants on fluorescence. Overall, the similarity of fluorescence EEMs, and also between PARAFAC components (Stedmon and Markager, 2005a; Murphy et al., 2008), observed in a wide range of natural and engineered and engineered environments, from saline marine waters (Coble et al., 2007) through to dissolved metal rich groundwaters (Baker and Genty, 1999) and river and wastewaters, suggest that matrix and quenching effects may be relatively unimportant. Variability of organic matter fluorescence caused by variations in the concentration and source of fluorophore appear to dominate fluorescence EEMs and PARAFAC analyses as long as organic matter concentrations are below a certain threshold. Review of the literature suggests that this value is likely to be $\sim 20 \text{ mg L}^{-1}$ (Hudson et al., 2008).

Fluorescence instrumentation is continually being improved allowing production in ever smaller sizes at a cheaper price, whilst allowing the measurement of

Table 3 – Evaluation of the relative importance of matrix effects for implementing fluorescence as a monitoring tool.

Matrix effect	Typical advanced treated water values	Anticipated impact based on literature	Is this a reversible effect?	Importance for implementation of fluorescence as a monitoring tool
Inner filtering	0.5–6.6 mg L ⁻¹ as C (Table 1)	Unlikely to impact as less than 25 mg L ⁻¹ as C (Section 6.1)	N/A	No interference
Temperature	Close to that of the environmental T, assume 20 °C range	1% decrease in peak T and C fluorescence with every 1 °C rise in temperature (Section 6.2.1)	Yes	Fluorescence intensity will alter but can be corrected based on T measurements
pH	6–8 (Table 1)	0 to <30% increase for all peaks	Yes in the range pH 2–12	Unimportant if pH range small (<2 units)
Metals ions	Variable, depending on coagulant, industrial input to WWTP, pipe material	Studies report varying conclusions	Unknown	Further investigation required although studies on engineered systems have shown no effect
Oxidants	UV Ozonation	Peak C decreases Variable conclusions for Peak C, decrease for Peak T	No	Oxidants may represent a major source of variation in fluorescence, the impact depending on the carbon concentration/character and oxidation process utilised. Further investigation is required
	0–1 mg L ⁻¹ as Cl ₂ , Cl ₂ /DOC < 2 (Table 1) Ozone + photocatalysis	Potentially a Peak C increase due to fragmentation 70% Peak C decrease		

fluorescence at ever lower wavelengths. Studies on sewage-derived fluorescence are already being conducted with success in the field, using commercially available portable spectrophotometers (Baker et al., 2004). However, it must be considered that sensitivity levels are instrument dependent and are affected by the following: (1) light source, such as continuous or flash lamps or LED sources; (2) method used for wavelength selection, for example, monochromators to allow measurement across a range of wavelengths or filters that permit light through at a fixed bandwidth; and (3) the photomultiplier tube detector. Each instrument configuration will have particular advantages and disadvantages for specific applications. For example, more compact devices generally use filter based systems for greater sensitivity, limiting measurement to one or two fixed wavelengths. Hence, portable devices are typically designed for specific monitoring applications, for example, algal monitoring at a fixed emission wavelength of 685 nm (Beutler et al., 2002). It would therefore be advantageous to determine relevant wavelengths of interest for recycled water monitoring, in order to facilitate the development of simple portable instruments.

Calibration is also an important consideration in the implementation of fluorescence as a monitoring tool to ensure repeatability and reliability of results. Like all instrumental measurements, fluorescence spectroscopy is subject to instrument bias resulting in systematic errors. Only by the application of specific correction factors can comparisons between different instruments be undertaken (Resch-Genger et al., 2005; Holbrook et al., 2006). It has been noted that the correction factors having the most distinct impacts on spectral shape and intensity relate to excitation beam intensity and the detector response (DeRose et al., 2007). While desirable, the success of fluorescence spectroscopy as a monitoring tool is not reliant on the ability to compare between instruments. However, it will be important to be able to correct for instrument drift, which must be monitored and well managed.

8. Conclusions

This review has identified an urgent need for alternative monitoring techniques suited to recycled water systems in which very small differences in TOC concentration and character require detection. Fluorescence spectroscopic techniques have considerable potential as monitoring tools over traditional methods, including UV spectroscopy, due to their greater sensitivity and selectivity.

Fluorescence spectra of sewage impacted water have shown correlations with typical water quality parameters, most consistently with BOD; however, such correlations have yet to be examined in depth for highly treated recycled water. Indications are that significant proportions of fluorescent DOC will remain upon advanced treatment, allowing fluorescence spectroscopic analysis of product water. However, the distinctiveness and stability of the fluorescence signature for recycled water in comparison to treated effluent and drinking water requires investigation. Similarly, the most appropriate method of data analysis needs to be determined. Many data analytical techniques have been discussed and research is

required to determine the most applicable in the implementation of fluorescence as a monitoring tool.

A significant challenge in the use of fluorescence as a monitoring technique is in overcoming matrix effects which alter the fluorescence signature. Such effects could mask small differences in TOC concentration and character that might otherwise be indicative of contamination. In particular, this review has highlighted the need for investigating the impact of oxidants on recycled water fluorescence. Additionally, calibration issues will need to be addressed, particularly with respect to accounting for instrument drift.

Overall, there is potential for fluorescence to be a successful tool for monitoring recycled water systems and technology is available to be used. A greater scientific understanding of fluorescence in recycled water systems is now required in order to successfully implement such technology and provide improved management systems for water recycling schemes, thus ensuring that public confidence is maintained.

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