The Use of Tryptophan-like Fluorescence as an Indicator of Organic Pollution

Fluorescence has long been used to measure a range of water quality parameters, from chlorophyll and algae to hydrocarbons and optical brightening agents. Recent advances in LED technology have led to the development of portable, submersible fluorimeters that can look further than ever before into the ultra-violet region, with some very interesting results…

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Background

Fluorimeters have been used for many years in the field of water quality monitoring and are the established and trusted technique for reliably measuring dissolved organic matter (DOM), chlorophyll and algal biomass. Fluorimeters work by emitting light at one wavelength and detecting light emitted by the target molecule at another wavelength. Only certain substances exhibit this property and at very specific pairs of wavelengths – this means that fluorescence can be a very selective and sensitive optical technique.

DOM in freshwaters consists predominately of decaying plant matter (humic or fulvic substances). In many cases plumes of DOM in a water body will also be accompanied by an active microbial community. It is this microbial community that consumes oxygen – leading to high levels of biological oxygen demand (BOD) and the subsequent crashes in oxygen levels that are detrimental to aquatic ecosystems. Proteins found in the cell walls of these micro-organisms have been shown to fluoresce in the same region as the amino-acid, tryptophan. Thus, 'tryptophan-like' fluorescence can be used as a measure of the microbial health of a water body and therefore as an indicator of BOD.

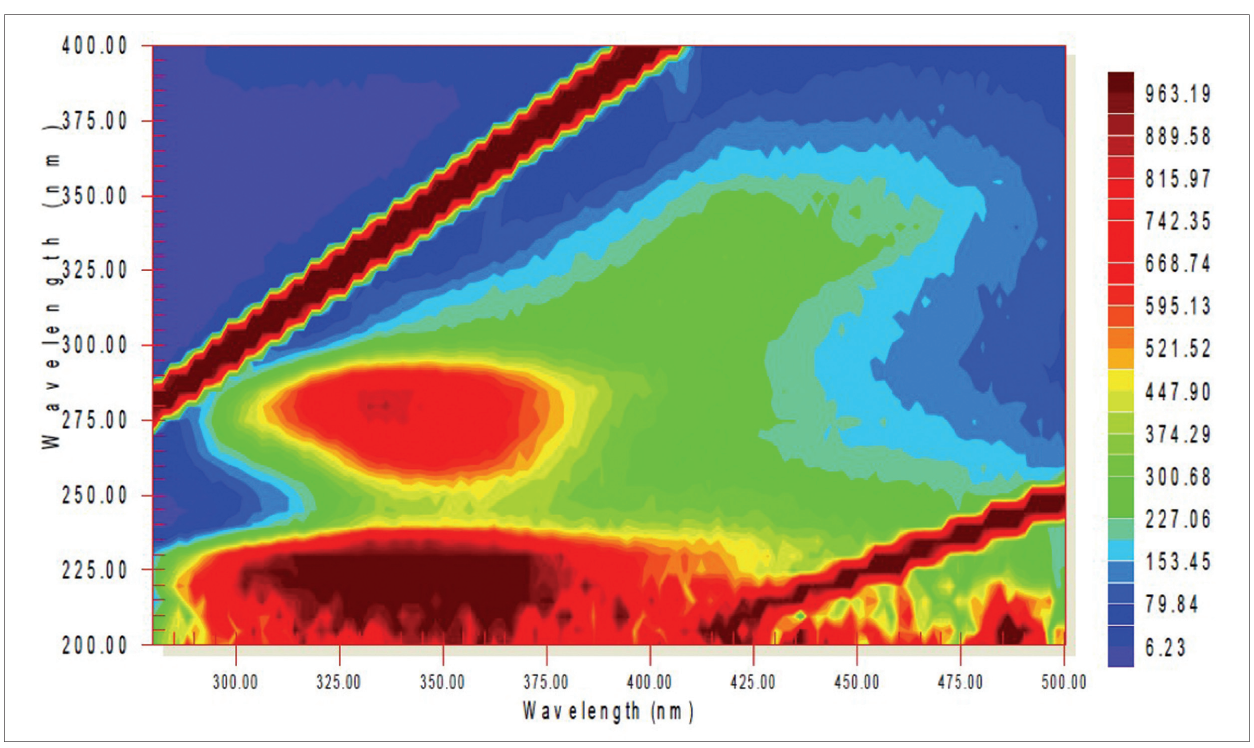
Common sources of polluting DOM such as silage liquor, cattle and pig slurries and human sewerage all fluoresce when excited at the same short ultra violet wavelengths (~280nm). This means that fluorimeters 'tuned' to this wavelength could be a uniquely useful tool for a wide range of monitoring applications in both rural and urban catchments.

What's new?

The original research that identified the different constituents of the fluorescence EEM was carried out in the laboratory using a sophisticated bench-top scanning fluorimeter. Once the specific excitation and emission wavelengths of the tryptophan-like peak (T) were identified then lower cost portable instruments could be developed that focused solely on that excitation/emission pair (i.e. 280nm/340nm). Due to the short wavelengths required to provide the correct excitation, high power xenon flash lamps were used as the source in early portable tryptophan-like fluorimeters. This meant that not only were they relatively large and expensive, but submersion was not possible – instead the sample was introduced via a quartz cuvette. The breakthrough recently occurred when light emitting diodes (LED) were developed that could attain the short wavelengths required. This now means that compact, submersible fluorimeters tuned specifically for tryptophan-like fluorescence can be produced in the same format as established sensors for chlorophyll, algae or optical brightening agents (OBA).

A Practical Solution

For the first time, robust and submersible tryptophan-like fluorimeters can now be deployed as an integral part of the flexible Manta 2 multi-sonde platform from Eureka. This opens up a whole new range of applications for exploiting the power of this novel technique. As the Manta 2 sonde can incorporate up to 12 sensors it will enable



Fluorescence excitation-emission matrix or EEM. Showing the strong peak T fluorescence centered around 280 nm excitation and 340 nm emission. Y-axis is excitation, x-axis is emission.

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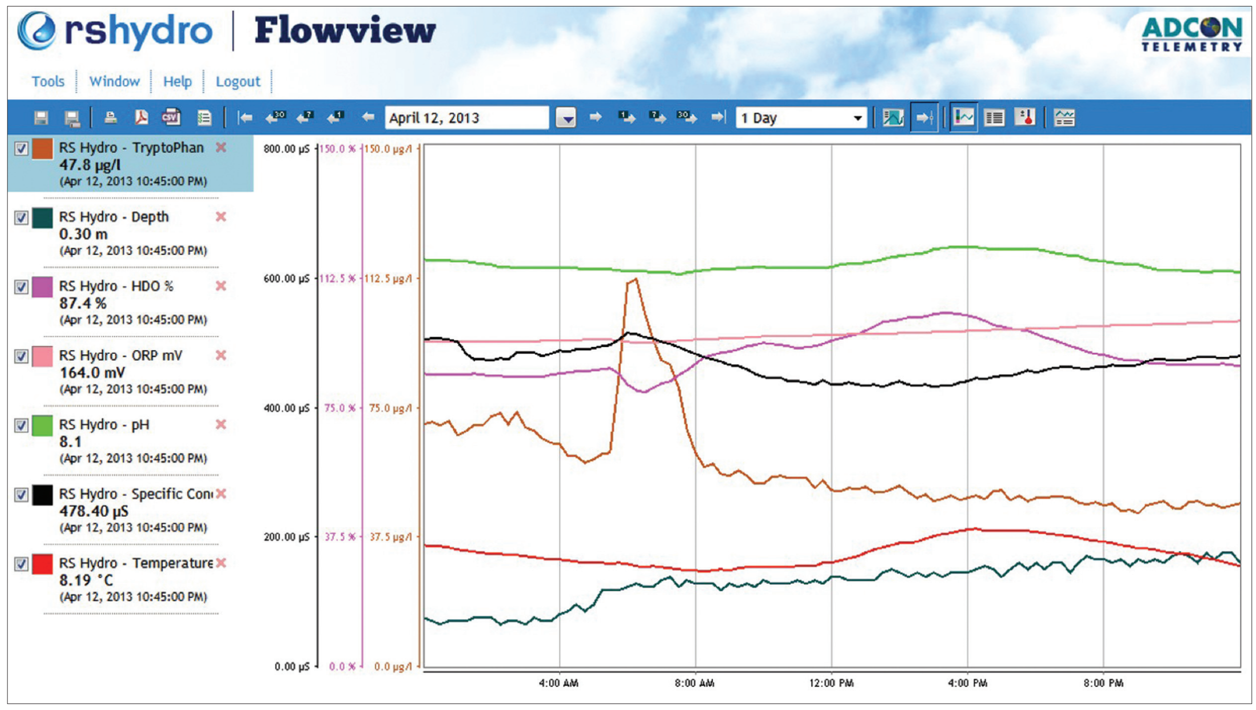
Eureka Manta2 – can now incorporate fluorimeters tuned for Tryptophan



Water quality trend downstream of a CSO – note the significant increase in Tryptophan-like fluorescence but only a small change in DO/Conductivity

traditional determinants of water quality, such as dissolved oxygen, pH and turbidity, to be measured continuously alongside tryptophan-like fluorescence. This will give a clearer picture of the impacts of polluting organic matter on the health of a water body, as well as a much better understanding of the relationships between DOM and more conventional parameters. The robustness, ease-of-use and comparative low cost of the submersible platforms take measurement of tryptophan-like fluorescence out of the hands of expert researchers and academics and into the hands of environmental practitioners with a limited level of technical expertise.

The Manta2 platform is easily interfaced to RS Hydro's Adcon wireless telemetry system – so that fluorescence data – alongside other parameters – can be collected remotely and in near real-time. The power and flexibility of the Adcon system and web based FlowView interface enables automatic water samplers and other devices to be triggered according to pre-determined levels of any parameter in the system.

The first tryptophan-like fluorimeter was recently fitted to a Eureka Manta2 sonde alongside sensors for temperature, optical dissolved oxygen, pH, oxidisation reduction potential (ORP), conductivity and depth.

Potential Applications

The facility to embed multiple water quality sensors – including tryptophan-like fluorimeters into a single field deployable platform opens up a range of new applications as well as offering improved tools for some commonly monitored situations. For example, tryptophan-like fluorescence could be a much more direct measurement of discharging combined sewage overflows (CSO) than using a combination of dissolved oxygen, ammonium and conductivity or pH sensors. The tryptophan sensor gives a distinct, positive signal during a CSO spill which is much better suited to triggering alarms than a combination of parameters that may not, in isolation, give a distinct response.

Tryptophan-like fluorescence is also likely to identify inputs of polluting organic matter earlier and more decisively than a suite of sensors designed to measure the effects of DOM. Depending on the type of organic matter, it can sometimes take a while for oxygen levels to drop and for elevated ammonium, nitrate or phosphate levels to develop. This lag-time translates into increased uncertainty over the location and timing of the pollution source. By directly measuring the fluorescent proteins in the cell walls of the microbial population, tryptophan-like fluorescence can also give an indication of the potential severity of subsequent effects of the pollution.

Early trials with the tryptophan sensor show that there is potential for it to be used in more industrial applications. Industries and processes that deal with meat, fish and dairy products often have waste streams that are very high in proteins. This waste is usually discharged into the sewerage system where treatment costs are apportioned according to infrequent sampling and analysis for either BOD or chemical oxygen demand (COD). These tests can be costly in themselves and are unable to provide the high temporal resolution needed to accurately monitor complex processes. The use of tryptophan-like fluorescence sensors for real-time monitoring of waste streams could not only enable better estimation of inputs to treatment plants, but also enable real-time optimisation of active treatment systems, thus reducing costs and penalties associated with under or over-treatment. There is some work to be done in order to optimise anti-fouling for these harsher conditions but fluorimeters are commonly fitted with brushes or wipers for long term deployments in surface water environments.

Perhaps the most exciting aspect of the miniaturisation of tryptophan-like fluorimeters is the ability to combine multiple sensors in the same platform and gain real-time, continuous data from complimentary sensors. The possibilities are too numerous to mention but already researchers and catchment management professionals are starting to think about how tryptophan-like fluorescence relates to dissolved oxygen levels and whether concurrent chlorophyll data will enable greater understanding of primary production processes. Simultaneous measurement of tryptophan levels and optical brightening agents (OBA are highly fluorescent and are commonly used in household cleaning products and are considered a ubiquitous tracer of human sewage) should enable the origin of polluting DOM to be established. For example – if a pollution event causes high levels of tryptophan and OBA then a human source is implicated, if no OBA are present then it is more likely from an animal or agricultural source. This could be a very useful tool for catchment management and for investigating the cause of Bathing Waters Directive failures.

Early Results

The first tryptophan enabled multiprobe has been deployed in a variety of situations over the past few months including ponds, rivers, small streams and industrial process streams. The data collected from these deployments has provided fascinating insights into the variability of tryptophan-like fluorescence during both storm events and base flow conditions. For example, during deployment in a small rural stream, a marked increase in tryptophan fluorescence was observed after a rainfall event following a dry period. Whilst concurrent readings of dissolved oxygen, pH and conductivity displayed only minor perturbations, the tryptophan sensor has detected a significant increase in tryptophan fluorescence which was linked to a CSO upstream.

Future Directions

Initial field trials are very promising with sensor readings being very repeatable and only requiring a relatively infrequent calibration procedure. Although initial results suggest sensor stability lasting many months, further work is needed to assess longer term sensor stability (e.g. sensor drift and power consumption) and behaviour of the Tryptophan-like fluorescence signal under differing water temperature and turbidity regimes. To this end a knowledge transfer partnership between RS Hydro and the University of Birmingham has been established with longer term field trials in both rural and urban catchments planned for the next two years to rigorously assess the sensor capabilities under a range of hydrological conditions. Furthermore, exploration of the relationship between the Tryptophan-like fluorescence signal and the 3-dimensional EEM will improve understanding of the processes driving signal variability. This work will further validate the combined sensor platform (i.e. tryptophan-like fluorimeter fitted to a Eureka Manta2 sonde) as a unique tool for monitoring and tracing polluting DOM sources.