Development and deployment of a microfluidic platform for water quality monitoring

John Cleary*, Damien Maher, and Dermot Diamond

CLARITY Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Dublin, Ireland

*Email: john.cleary@dcu.ie

Abstract

Abstract. There is an increasing demand for autonomous sensor devices which can provide reliable data on key water quality parameters at a higher temporal and geographical resolution than is achievable using current approaches to sampling and monitoring. Microfluidic technology, in combination with rapid and on-going developments in the area of wireless communications, has significant potential to address this demand due to a number of advantageous features which allow the development of compact, low-cost and low-powered analytical devices. Here we report on the development of a microfluidic platform for water quality monitoring. This system has been successfully applied to in-situ monitoring of phosphate in environmental and wastewater monitoring applications. We describe a number of the technical and practical issues encountered and addressed during these deployments and summarise the current status of the technology.

Keywords Microfluidics, autonomous sensing, chemical sensors, water quality

1. Introduction

There is a growing need for increased monitoring of water quality across a broad range of applications, including municipal and industrial wastewaters as well as drinking water and environmental waters (rivers, lakes, groundwater and marine). This demand is fuelled by a range of drivers, with legislation being the most direct and important in many applications. For example, the European Union’s Water Framework Directive (WFD) [1], supported by a range of directives such the Urban Wastewater Directive [2], the Nitrates Directive [3], the Drinking Water Directive [4] and the Groundwater Directive [5], establishes a comprehensive basis for the management of water resources within the EU. The priorities of the WFD are to prevent further deterioration of and to protect and enhance the status of water resources and to promote sustainable water use based on long-term protection of water resources. To this end, the Directive requires that an integrated monitoring programme be established within each river basin district, which will enable collection of the physical, chemical and biological data necessary to assess the status of surface and groundwater bodies in each such district.

Reliable, high quality, and high resolution information about water quality is essential for water management and for improving the environmental quality of water resources. However, existing monitoring practices are unsatisfactory for a variety of reasons. Monitoring of environmental waters is still predominantly based on manual sampling followed by laboratory analysis using standard techniques such as colorimetry, atomic absorption spectroscopy, mass spectrometry and ion chromatography. While this approach yields high quality and reliable data (assuming that the appropriate protocols are followed during collection, transport, storage and analysis of the sample) the cost per sample is significant due to the manpower requirement for sample collection as well as the cost of analysis. This approach is therefore incapable of meeting the demand for monitoring at the much higher temporal frequencies and geographical densities which are envisaged [6].

For wastewater monitoring, a range of online sensors and analysers are commercially available, based on colorimetric chemical detection, electrochemical detection or optical detection. Generally available as single units or a suite of units aimed at monitoring individual parameters such as phosphate, nitrate or ammonia, these are also subject to a number of limitations, including: high cost of purchase and installation, high cost of operation due to the significant volumes of reagents and/or standard solutions required, and varying reliability, leading to frequent intervention by operators in order to clean fouled surfaces, remove blockages, or run calibration protocols. In this sector, the key advantage of microfluidics-based sensing systems is the lower cost of purchase and operation, as well as reduced infrastructure requirements. For this reason, microfluidic systems are likely to find a significant niche in the wastewater sector, particularly in smaller-scale wastewater treatment plants where the cost of conventional online systems may be prohibitive, and in alternative treatment systems such as constructed wetlands [7] where mains power may be unavailable or restricted to parts of the site. The installation of large numbers of new wastewater treatment systems in developing economies also represents a major opportunity for developers of low-cost monitoring systems for key parameters such as nutrients.

Since the “miniaturised total analysis system” (µTAS) concept was introduced by Manz et al. in 1990 [8], it has been recognised that the properties of microfluidic or “lab-on-a-chip” systems make them eminently suitable for the development of compact, autonomous analytical devices for a range of applications including environmental monitoring. The advantages of microfluidic sensing systems can be summarised as follows. (i) The small sample sizes used, typically in the µL range, minimise reagent consumption and waste generation. (ii) The small size of the microfluidic manifold facilitates the development of compact and portable analytical systems. (iii) Fast analysis times result from performing chemical analysis on the µm scale, where diffusion-based mixing can be an efficient process, allowing high sample throughput and/or frequent measurements. (iv) Low-cost sensing devices can be developed by combining microfluidic systems with simple, low cost detectors. This combination of properties makes microfluidic systems highly attractive as a basis for reagent-based monitoring of chemical species in the aquatic environment.

There are also drawbacks associated with operating at the micro-scale. Using µL-scale sample sizes has implications in terms of adequately representing the complete body of water which is to be measured. This issue is significantly mitigated by the higher measurement frequency which is possible using *in-situ* systems; for example, by capturing short-term variations, multi-daily *in situ* measurements which are approximately accurate will provide more information on the levels of a target analyte in a water body than a highly accurate sample-based measurement which is only carried out at monthly intervals. Due to the small dimensions of the microfluidic channels, they are susceptible to blockage or interference by fine particulate matter. The use of fine filters at the sampling point can eliminate or minimise this issue, although at the cost of limiting the analytical parameter to the dissolved fraction of the target species. Issues such as these, and others including interference caused by bubble formation/trapping within the micro-analytical system, have represented significant barriers to the development and uptake of microfluidics-based *in-situ* sensing systems. Although numerous micro-analytical systems have been developed and their performances described under laboratory conditions, examples of integrated micro-analytical devices which have been successfully deployed for extended periods under real environmental conditions are much scarcer. Therefore, while the development of sensing nodes for the aquatic environment which are sufficiently reliable and low in cost to form the basis of extensive wireless sensor networks has been anticipated, realisation of this goal has been more difficult to achieve. The challenges in developing such systems include achieving adequate stability and reliability of the analytical system, cost and power consumption of the integrated device, robustness under harsh environmental conditions, and fouling due to particulate matter and microorganisms. In developing an autonomous environmental monitoring device, deployable lifetimes on the order of months to a year are desirable. Achieving such lifetimes without elevating the fabrication cost of the integrated system to unviable levels can be described as a key challenge for the analytical science community [9].

2. Examples of deployable microfluidic devices

A range of reagent-based analytical techniques can be readily implemented in microfluidic systems. However, despite rapid and on-going developments in the area of microfluidic/lab-on-a-chip technology for chemical analyses over the last 20 years or so, and the significant requirements and opportunities which exist for autonomous environmental monitoring systems [10,11], relatively few field deployable microfluidic devices based on reagent chemistry have come to fruition.

One area which has seen significant progress is the integration of colorimetric methods for nutrients such as phosphate, ammonia and nitrate/nitrite into microfluidic manifolds with light emitting diode (LED)/photodiode-based optical detection systems. Gardolinski *et al.* [12] developed a miniaturised, LED-based chemical analyser for *in-situ* monitoring of nitrate. The limit of detection (LOD) with a 20 mm path length flow cell was 2.8 mg L^{-1} N. The linear range could be adjusted to suit local conditions in the field by changing the flow cell path length. The analyser was tested in shipboard mode for mapping nitrate concentration in the North Sea and in submersible mode to conduct a transect of Tamar Estuary (England).

Doku *et al.* [13] developed a micro-flow injection analysis (µFIA) technique for orthophosphate based on the molybdenum blue reaction. The µFIA manifold was formed by etching of borosilicate glass, and electroosmotic flow was used both for the mobilization of reagents and for sample injection. A LOD of 0.1 µg mL^{-1} was achieved, with a rapid analysis time of 60 s and low sample/reagent volume (total system volume of 0.6 µL). Greenway *et al.* [14] produced a similar µFIA system for the determination of nitrite, based on the Greiss reaction to form an azo dye. Following optimisation of the electro-osmotic flow characteristics, reaction chemistry, and injection time, a LOD of 0.2 µmol L^{-1} was achieved. A further progression of this work was to incorporate a cadmium reduction column within the device. This allowed for the analysis of nitrate in water, for which a LOD of 0.51 µmol L^{-1} [15] was achieved. Daridon et al. [16] investigated the Berthelot reaction for the determination of ammonia in water utilising a microfluidic device consisting of a silicon chip between two glass plates. The pathlength in this device was 400 µm, and the integrated system comprised fibre optics coupled to an LED. High aspect ratio channels (30 µm wide × 220 µm deep) were used for sample/reagent mixing in order to achieve efficient diffusional mixing.

Azzarro et al. [17] described an automatic colorimetric analyser prototype (MicroMAC FAST MP3) for high frequency measurement of nutrients in seawater. This system utilises the Berthelot method for ammonia detection, the sulphanilamide/ethylendiamine method for nitrate detection, and the blue phosphomolybdate method for phosphate detection, and LODs of 5, 2.5 and 2.5 µg/L were reported for $N\text{-}NH_4$, $N\text{-}NO_3$ and $P\text{-}PO_4$ respectively. The system was based on loop flow analysis technology developed by Systea, an Italian company, and has been further developed to provide a system more suited to deployment on typical monitoring platforms. This multi-nutrient analyser system has been trialled in collaboration with YSI Hydrodata at two locations in the United Kingdom (Hannigfield Reservoir and River Blackwater Estuary) [18].

A number of other systems for *in-situ* nutrient monitoring have been assessed by the Alliance for Coastal Technologies (ACT) at Chesapeake Biological Laboratory, USA [19-21]. ACT conducts technology evaluations which focus on classes of commercially available instruments in order to provide confirmation that each technology meets the manufacturer's performance specifications or claims under real deployment conditions. The American Ecotech NUT 1000 was originally developed at Monash University and commercialised by Ecotech Pty. Ltd. The system can achieve LODs for reactive phosphate of < 3µg L^{-1} with a response time of 30 seconds. The high sampling rate is allowed by the use of rapid sequenced reagent injection in combination with a multi-reflection flow cell. Reagent injection also minimises the reagent consumption, allowing over 1000 measurements to be performed with only 20 mL of reagent. In the ACT trials, the NUT 1000 was used for surface mapping on board a research vessel in Monterey Bay, California [19]. Very good correlation with validation samples was achieved, after correction for a measurement offset of 50 µg L^{-1} $P\text{-}PO_4$, which was attributed to differences in refractive index of natural seawater versus the reagent grade water used for preparation of internal standards and machine calibration. The ACT has also reported on successful deployments of the WET Labs Cycle-P nutrient analyser [20] and the YSI 9600 Nitrate Monitor [21]. Vuillemin *et al.* [22] described a miniaturised chemical analyser (CHEMINI) for dissolved iron and total sulphide, based on FIA and colorimetric analysis. Designed for deep-sea applications, the system is submersible to a depth of 6000m, with detection limits of 0.3 µM and 0.1 µM for iron and sulphide respectively. It was used to monitor the chemical environment within mussel beds, over a 6 month deployment period during which 8 measurements per day were performed.

Mowlem and co-workers at the National Oceanography Centre, Southampton, UK, have developed *in-situ* sensors for a range of chemical parameters [23-27] including nitrite, nitrate, ammonia, phosphate and iron. The first of this series of microfluidic chemical analysis systems [23] was used to detect nitrate and nitrite with a limit of detection (LOD) of 0.025 μM for nitrate (0.0016 mg L^{-1} as NO_3^-) and 0.02 μM for nitrite (0.00092 mg L^{-1} as NO_2^-). The device was deployed in an estuarine environment (Southampton Water, UK) to monitor nitrate and nitrite concentrations in waters of varying salinity and was able to track changes in the nitrate-salinity relationship of estuarine waters due to increased river flow after a period of high rainfall. In subsequent work, nanomolar detection limits were achieved for iron [24], ammonium [25], phosphate and nitrate [26]. Like the CHEMINI system, these analysers are designed primarily for oceanographic applications, and the level of engineering required to achieve the high analytical performances required in this environment means that the cost of such systems is likely to be prohibitive in terms of deploying large numbers of devices for routine monitoring applications.

3. Reagent stability

In many cases, reagent stability represents a major limitation when considering chemical detection methods for environmental monitoring, due to the need for devices to be exposed to environmental conditions for a significant period of time (months or longer) while giving reliable and reproducible results over this time frame. Sequeira et al. [28] examined the stability of the reagents for the Berthelot reaction for ammonia detection in terms of viability for long-term monitoring applications. The three key reagents in the reaction are potassium sodium tartrate, phenol and hypochlorite. While potassium sodium tartrate and phenol are generally regarded as stable if stored properly, hypochlorite solutions are unstable and can decompose to form chlorate ion and oxygen over a period of days or weeks. It was shown that iron and copper, present as contaminants, catalyse the decomposition. Ensuring that the hypochlorite solution was free from these contaminants allowed long-term storage of the hypochlorite solution without significant loss in activity. Salicylate was also used as a replacement for phenol, and was found to yield broadly similar analytical performance in terms of the sensitivity, and kinetics, while having the major advantages of being highly stable and non-toxic. Diamond and co-workers [29,30] evaluated the yellow vanadomolybdophosphoric acid method as an analytical method for the determination of phosphate in water within a microfluidic device with the aim of producing an automated device with a field-deployable lifetime of one year. In this method ammonium molybdate, $(NH_4)_6Mo_7O_{24}.7H_2O$, is reacted with ammonium metavanadate, NH_4VO_3, under acidic conditions. The combined reagent and sample containing orthophosphate react to form the vanadomolybdophosphoric acid complex, $(NH_4)_3PO_4NH_4VO_3.16MoO_3$. The resulting solution has a distinct yellow colour arising from the strong absorbance of this complex below 400 nm. It was shown that batches of the reagent could be used for over a year without significant loss in performance [29]. This method was therefore selected in preference to the molybdenum blue method, due to the greater stability of the reagents used in the analysis, and resulted in an assay with an LOD of 0.2 mg L^{-1} and a dynamic linear range of 0–50 mg L^{-1}.

4. First generation autonomous microfluidic system for phosphate analysis

Following on from investigations on the microfluidic application of the vanadomolybdophosphoric acid method for phosphate detection, Diamond and co-workers have developed an autonomous microfluidic analyser for phosphate, which has been successfully deployed in wastewater, freshwater and estuarine locations. An early prototype for this system consisted of bottles for storing the reagent, calibration solutions and cleaner, a sample port for collecting the water sample to be analysed, and an array of solenoid pumps for pumping the required liquids through the microfluidic chip. The microfluidic chip allowed for the mixing of the reagent and sample, and chip also presented the reacted sample to a photodiode and LED for an absorbance measurement. The analysed sample is then pumped to the waste storage container. All of the fluid handling and analytical components were controlled by a microcontroller that also performed the data acquisition and stored the data in a flash memory unit. A GSM modem was used to communicate the data via the SMS protocol to a laptop computer. The layout of the new system is shown schematically in Fig. 1.

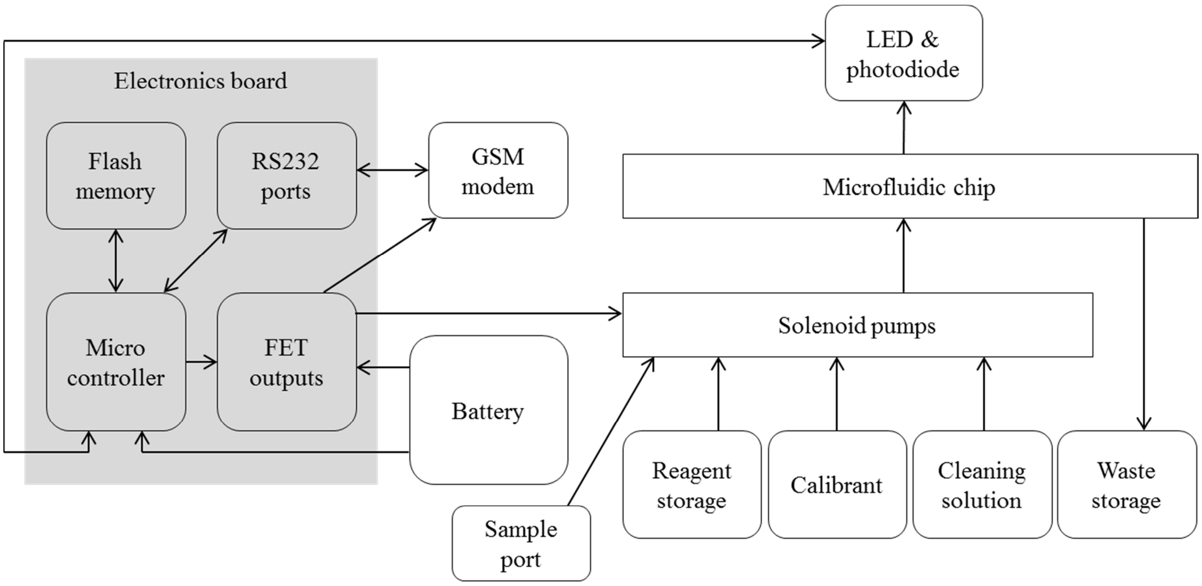

Fig.1. Schematic detailing the components of the phosphate sensor and their relation to each other.

The microfluidic chip used to perform the colorimetric measurement is shown in Fig. 2(a). This allows for the colorimetric reaction to be performed using 20 µL each of sample and reagent. The reagent and sample are mixed in a T-mixer which leads into a serpentine channel where the resulting solution is presented to the emitter and photo-detector for the absorbance measurement. Each side of the T-mixer has three inlet channels leading into them. On one side are the two phosphate standard solutions for a two point calibration. A third inlet channel is for the water sample. On the other side of the T-mixer there is an inlet for the reagent and two inlets for deionized water for cleaning the microchannels. All channels are of 200µm in width and depth. The chip was manufactured from a 1 mm thick PMMA sheet (ME303011, Goodfellow, Cambridgeshire, England). The microfluidic channels were milled into the sheet using the CAT-3D-M6 micromill (DATRON Electronic, Mühltal, Germany) using a 200µm endmill (00680029, DATRON Electronic, Mühltal, Germany). The 35×20 mm chip was then cut from the sheet. The channels are sealed by bonding a second 35×20 mm PMMA chip layer using a pressure sensitive adhesive (ARcare® 8890, Adhesives Research, Limerick, Ireland). PEEK tubes are inserted into the inlet and outlet ports and are held in place using epoxy adhesive.

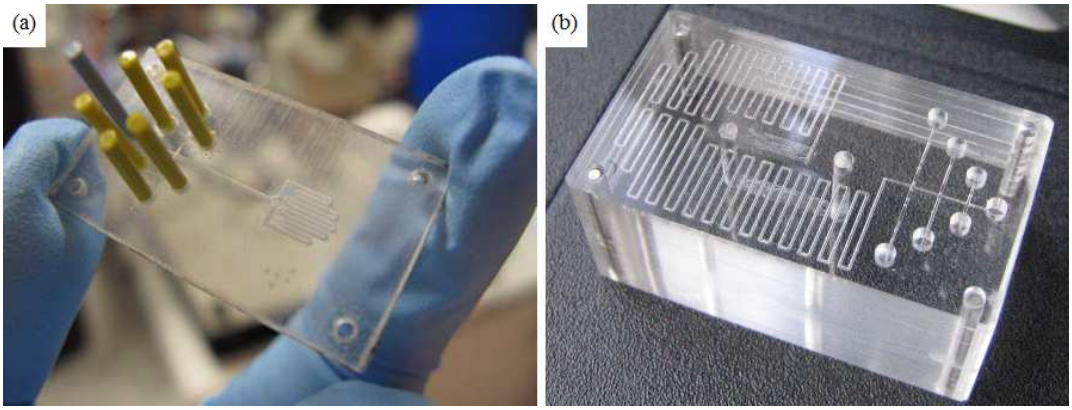

Fig. 2. Two versions of the microfluidic chip used for phosphate detection. (a) The prototype microfluidic chip described in this section, with serpentine channel visible on the right of the chip; the six inlets and single outlet can be seen on the left. (b) A redesigned version of the chip in which a serpentine channel was used for mixing and reaction of sample and reagent, and a cylindrical optical cuvette replaced the serpentine detection area.

The reagent, calibration solutions and cleaners were stored in five square 250 ml HDPE bottles (NALGENE 2018-0250, Nalge Nunc, New York, USA). This capacity of reagent storage allowed for over 11,000 phosphate measurements. Two square 1L HDPE bottles were used for waste storage (NALGENE 2018-1000, Nalge Nunc, New York, USA). The bottle caps (Q-Series, Bio-Chem Valve, New Jersey, USA) used were fitted with Teflon check valves, which allowed air pressure to equalize between the bottle and its surroundings as the liquid is being pumped out of or into the various bottles. Teflon tubing with ¼”-28 UNF connectors (Omni-Lok, Bio-Chem Valve, New Jersey, USA) fitted at the ends, were used to connect the various fluidic components in the system. Six independently controlled miniature solenoid pumps (120SP, Bio-Chem Valve, New Jersey, USA) were used to control fluid movement in the system. The fluid-contacting parts of these pumps are made from chemically PEEK for high chemical resistance. The pumps deliver 20 μL for each stroke and have ¼”-28 UNF ports for tubing interconnects. The system used dedicated pumps for the water sample, two calibration solutions, and the reagent. The remaining two pumps were used to deliver a cleaning solution to the microfluidic chip between assays.

The microcontroller used for the control of the system was the MSP430F449 (Texas Instruments), which was chosen for its low power consumption during operational and sleep mode. The MSPGCC compiler also provided an open source platform for software development (mspgcc.sourceforge.net). This microcontroller has, along with numerous digital inputs and outputs, an 8 channel 12-bit ADC for data acquisition and two UART ports for communicating with peripheral devices. A 2 megabyte SPI flash chip mounted on the PCB with the microcontroller allowed for 16,384 data points to be logged. The solenoid pumps and the power to the GSM modem were controlled via an array of field effect transistors (FETs). A photodiode (S1227-33BR, Hamamatsu Photonics UK Limited, Hertfershire, UK) and a 370nm LED (NSHU550E, Nichia Corporation, Tokushima, Japan) were used for the absorbance measurement. A transimpedance amplifier circuit based around a TLV2772 operational amplifier (Texas Instruments) was used to condition the signal from the photodiode. This circuit was built on a separate PCB from the main controller board, in order to limit the noise on the signal from the photodiode, which is mounted beside the microfluidic chip. A temperature sensor (LM335, ST Microelectronics) is also mounted on the daughter board. The LED is controlled via a BJT and was pulsed during the absorbance measurement to reduce power consumption and extend LED lifetime. The voltage signals from the transimpedance circuit and the temperature sensor were read by the ADC on the microcontroller. The entire system was powered by a 12V 7Ah standard lead acid battery as used in the previous system. The power source was chosen for its low cost, long lifespan and high capacity. The microcontroller also monitored and logged the battery voltage with each phosphate measurement.

A GSM modem was used for communication during system deployments. The main reason for the choice of this communications standard was the extensive coverage of the GSM network in Ireland and other countries in the European Union, which meant that the phosphate sensor could be deployed in most areas while maintaining data communication capability. The modem used was the MC35IT (Siemens, Berlin, Germany) which communicates via RS232, allowing for easy implementation in the system. The system used the GSM modem’s SMS functionality to send the data to another GSM modem connected to a laptop computer which acted as a base-station for data collection. During the operation of the sensor, data was collected and then stored in the flash memory. After a predetermined number of measurements the collected data was transmitted via SMS messages to the base-station. To withstand the environment the sensing system was housed in a rugged enclosure, in the form of a polypropylene “Pelicase” (1430, Peli Products, Barcelona, Spain). This case is completely water tight and resistant to the reagent used. It is lightweight and so does not affect portability. The chemical storage bottles and battery were mounted in the lower part of the case, while the solenoid pumps and microfluidic chip and LED and photodiode were mounted on a custom ABS mounting plate manufactured using a 3D printer (Dimension SST768, Stratasys, Minnesota, USA). The controller board and GSM Modem were mounted in the enclosure lid. The sample port was mounted on the side of the enclosure.

Fig. 3 shows the fully assembled system with Pelicase enclosure. The five storage bottles and two waste bottles can be seen at the bottom of the enclosure. The battery sits between the waste bottles. The top plate is flipped up to reveal the six solenoid pumps on the underside. These are mounted with the inlets facing down towards the storage bottles. The outlets can be accessed through the top to allow them to be connected the microfluidic chip. This is housed along with the temperature sensor, LED and photodiode with their accompanying daughter board in the center of the top plate. In the lid of the enclosure the controller board is mounted on the left hand side. The GSM modem along with its antenna is mounted on the right. The sample is drawn into the system through a 0.45 µm pore diameter filter membrane (Supor, PALL Corporation, Michigan, US), held in place between two polycarbonate plates and sealed with O-rings (Fig. 4). The port is attached to the Pelicase and sealed with polyurethane adhesive. The port has a ¼”-28 UNF port for connecting tubing.

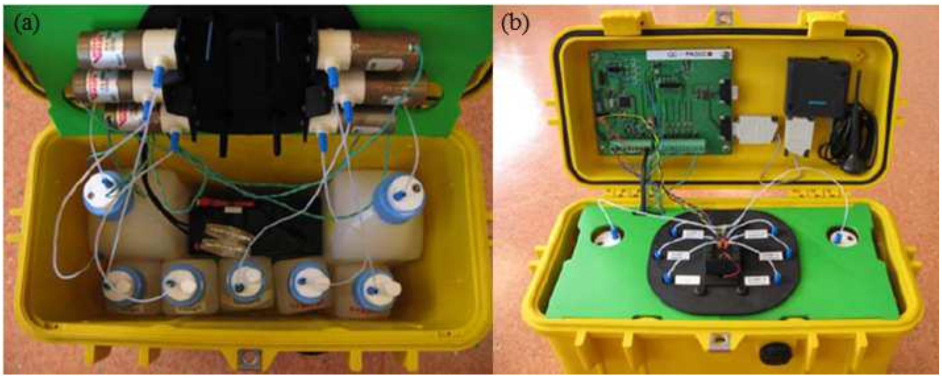

Fig. 3. Fully assembled system. (a) Bottom of enclosure showing bottles and battery. (b) Top plate and lid showing electronics board and GSM modem. Adapted with permission from [31].

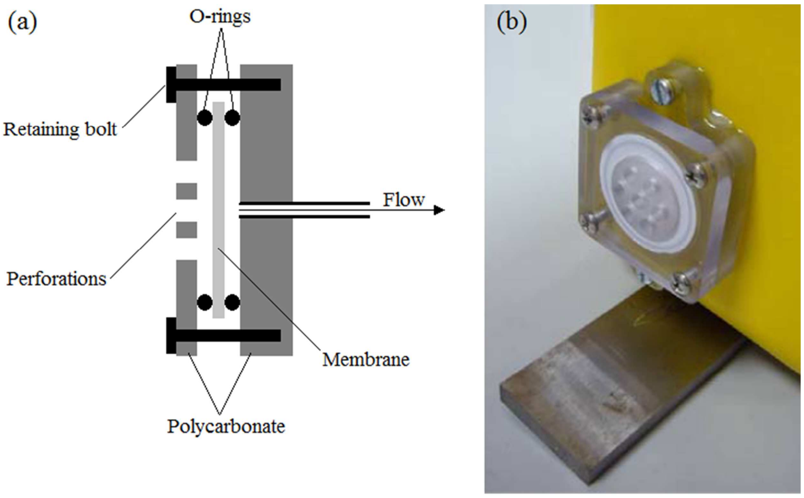

Fig. 4. (a) Schematic of sample port. (b) Sample port mounted on sensor housing. Adapted with permission from [31].

5. *In-situ* testing of first generation phosphate analyser

Following laboratory based validation studies [31,32], the first generation phosphate analyser was deployed at a wastewater treatment plant (Osberstown Wastewater Treatment Plant, Co. Kildare, Ireland) over a 6 week period in July and August 2008. The analyser was situated within the monitoring building which houses the WWTP’s online monitoring systems for phosphate, ammonia and nitrate. Sample was pumped from the final effluent tank adjacent to the monitoring building, and a valve was added to the sample line to deliver sample to the prototype phosphate analyser’s sample inlet. For the purposes of this trial, the sample inlet was modified to consist of a modified membrane filter holder (shown schematically in Fig. 5) which allowed sample to contact a membrane filter (Supor membrane filter, 0.45 µm pore size, 25 mm diameter, Sigma-Aldrich) and then exit to waste. Sample was drawn through the membrane and into the analyser system using a solenoid pump which then delivered the sample to the microfluidic chip.

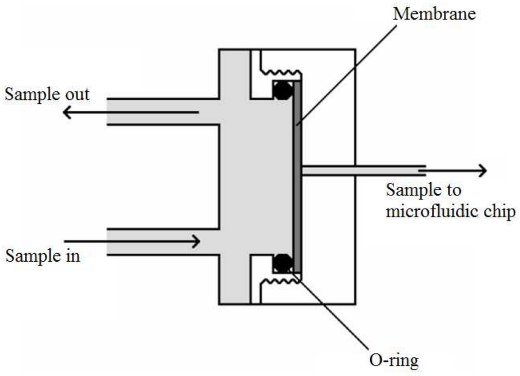

Fig. 5. Schematic cross section of the sampling port. The threaded parts of the membrane holder are screwed together so that the membrane is held in place by the O-ring seal. Sample circulates past the outer side of the membrane and the required volume is drawn into the analyser by a solenoid pump. Adapted with permission from [45].

In order to compensate for variation in the detector output due to diurnal temperature fluctuations, as well as signal drift over long time periods, a calibration procedure was automatically carried out for each sample measurement. This was performed by carrying out separate reaction and detection runs using blank (0 mg L^{-1} phosphate) and standard (10 mg L^{-1} phosphate) solutions. The absorbance of the standard phosphate solution was calculated using the formula:

$$A = log(I_o/I) \qquad \text{(Eqn. 1)}$$

where A is absorbance, I_o is the measured light intensity for the blank solution, and I is the measured light intensity for the standard solution. The absorbance for the sample solution was calculated similarly, and was converted to phosphate concentration using the absorbance of the standard solution. Finally, the sample's phosphate concentration value was converted to "phosphorus as phosphate" (P-PO_4) in order to facilitate comparison with the plant's existing online phosphorus monitor (Aztec P100 instrument, Capital Controls, UK). Data from the latter system was downloaded via serial connection to a laptop computer at weekly intervals.

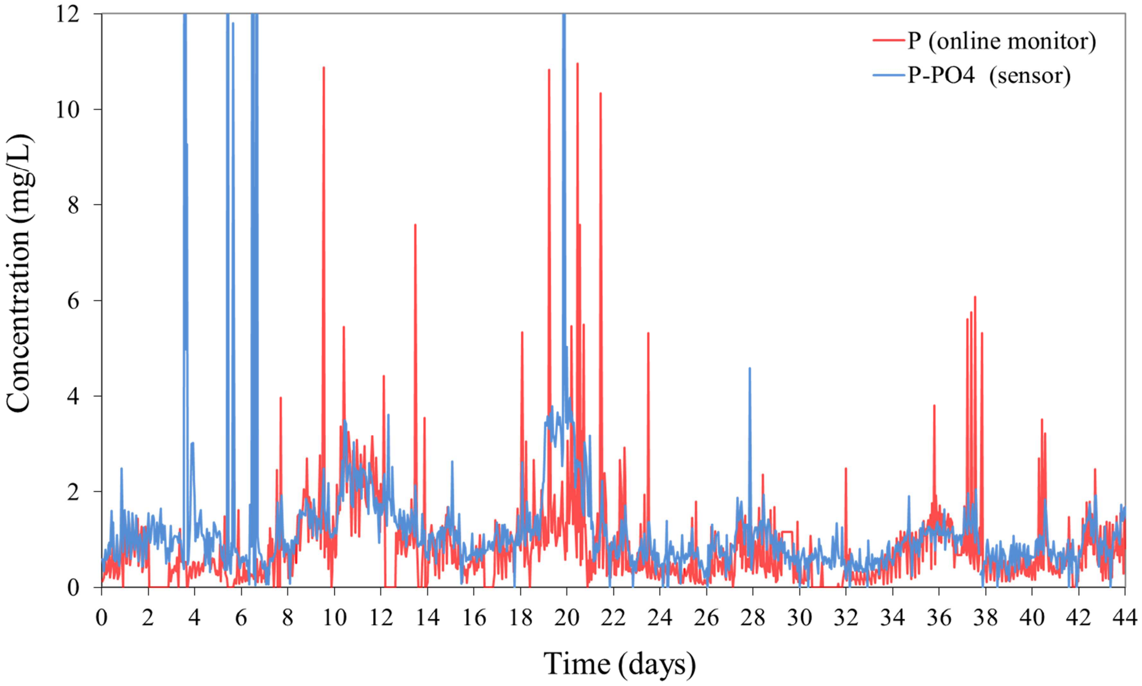

Fig. 6. Output from the prototype phosphate sensor and the plant's online phosphorus monitor during the trial period. Adapted with permission from [45].

The complete dataset from the 6 week trial is presented in Fig. 6. Over the period as a whole there is excellent agreement between the data collected from the prototype sensor and from the online monitoring system. However, a number of discrepancies were also observed. Between days 3 and 7 of the trial there were a number of spurious high readings in the prototype sensor data. These were caused by air bubbles accessing the external sample line and being drawn into the microfluidic chip where they interfered with the passage of light through the optical cuvette. On day 7 of the trial the valve on the sample line was adjusted which resulted in this issue being almost entirely eliminated, with only 2 further such readings observed (days 20 and 28). A more long-term deviation between the sensor and online monitor data was observed over days 18-21 of the trial, and was found to have been caused by the membrane filter becoming clogged with solids. After the filter was replaced, a close agreement in the data was resumed, and the membrane filter was changed at weekly intervals thereafter to prevent a recurrence of this issue. There were also a number of occasions on which the online monitor recorded elevated values which were not detected by the prototype sensor. These discrepancies were caused by solids in the sample affecting the online monitor readings, which has a relatively coarse filter at its inlet point. The online monitor uses heating and acid to digest solids in the sample, followed by analysis using the blue molybdate method for phosphate detection. Any solid material therefore has the potential to increase the measured value (acid-hydrolysable phosphorus). This is not the case with the prototype sensor, as solids are excluded from the system using the 0.45 μm filter membrane at the inlet point, in order to avoid blockage or fouling of the microfluidic channels. Despite the limitations involved in using the online monitoring data as validation for the prototype sensor, this trial data showed that the prototype phosphate sensor had considerable potential to be used for monitoring of phosphate in wastewater streams. The performance of the prototype sensor was shown to be comparable with that of a commercially available phosphorus monitor, while significantly less maintenance was required over the trial period, even after the membrane filter was changed at one week intervals to account for the heavy fouling potential of the sample. Running cost of the sensor was significantly lower than the online monitor, due to the minimal amount of reagent and calibrant solutions required, and the sensor’s much lower power consumption.

The first generation phosphate sensor has also been used to monitor phosphate levels in an estuarine environment in Co. Dublin, Ireland during two separate deployment periods in September-October and October-November 2009. Following a laboratory-based calibration procedure, the sensor was placed *in-situ* at Broadmeadow Water in Co. Dublin, Ireland on 25 September 2009. This is an estuarine water body which is known to have significantly elevated nutrient levels due to a combination of inputs from agricultural sources and wastewater treatment plant discharges [33]. The sensor was located at a small islet in one of the estuary channels and held in place using a steel anchoring device. As this was a tidal location, the GSM modem antennae was located outside the box and elevated above the high-water mark to ensure constant network coverage, as the sensor enclosure itself was fully immersed at high tide. The sensor operated with hourly measurement frequency, and data was transmitted by the GSM modem in SMS mode to a laboratory-based laptop computer at 5 hour intervals. For validation purposes, daily manual samples were collected as close as possible to the sensor’s sample inlet, and timed to coincide with the sensor’s sampling time. Samples were filtered immediately, and analysed using a Hach-Lange DR890 portable colorimeter and the appropriate reagent pack (amino acid method for high range phosphate).

Fig. 7. The phosphate sensor *in-situ* during a trial at Broadmeadow Water, Co. Dublin, Ireland in September 2009. The sample inlet is visible below the water line.

Fig. 8(a) shows the sensor's output during the trial period. The sensor performed 236 measurements during the period in question. A distinct daily pattern of variation emerged which is ascribed to tidal influences in the estuary. The sensor was located within 100 m of a wastewater treatment plant outfall, and changing tidal levels would significantly affect the degree of dilution which the wastewater discharges were subject to at a given time. Fig. 8(a) shows that there was generally good agreement between the phosphate concentrations measured by the sensor and by the validation method. The correlation coefficient (R^2) of 0.699 indicates that some discrepancies existed between the two datasets, despite the synchronous collection of manual samples from as close to the sensor inlet as possible. Given the rapid rate of change of the concentrations at certain times (Fig 8a), it is not surprising that such differences exist. Other possible sources of discrepancy include the small volume (22 μL) of sample used in the sensor-based assay, which may be less representative, at a particular moment in time, of the general water body than the 250 mL manual sample. Possible sources of error in the manual sampling data include minor inaccuracies in the sample volume used for analysis (25 mL) which, for on-site measurements, is measured using a graduated vial, or in the volume of the reagent solutions (ammonium molybdate solution and amino acid solution, 1 mL in each case). Perhaps more significantly, differing interfering species for the two methods could also give rise to some discrepancies. The molybdenum yellow method used in the phosphate sensor is subject to interference by dissolved organic substances with light absorption in the UV region. This was unlikely to be a significant factor in this study as after filtering to remove suspended solids, all water samples were observed to have good optical clarity without significant colour. Silica (Si), arsenate (AsO_4^{3-}) and ferrous iron (Fe^{2+}) are other potential interferants with the molybdenum yellow method [34]. Silica and arsenate, however, are mainly of concern when the reaction is heated to provide faster reaction rates, which was not the case in this study, and ferrous iron does not interfere at concentrations below 100 mg L^{-1}. The impact of interferants on the sensor data is therefore not likely to have been significant. Potential interferants for the amino acid method include sodium (Na^+), nitrite (NO_2^-) and sulfide (S^{2-}) [35]. The estuarine location used in this trial is a dynamic water body, affected by tidal fluctuations and a significant point source of phosphate and other nutrients (wastewater treatment plant discharge). Altering levels of these interferants over time have the potential to cause discrepancies between the sensor data and manual measurements.

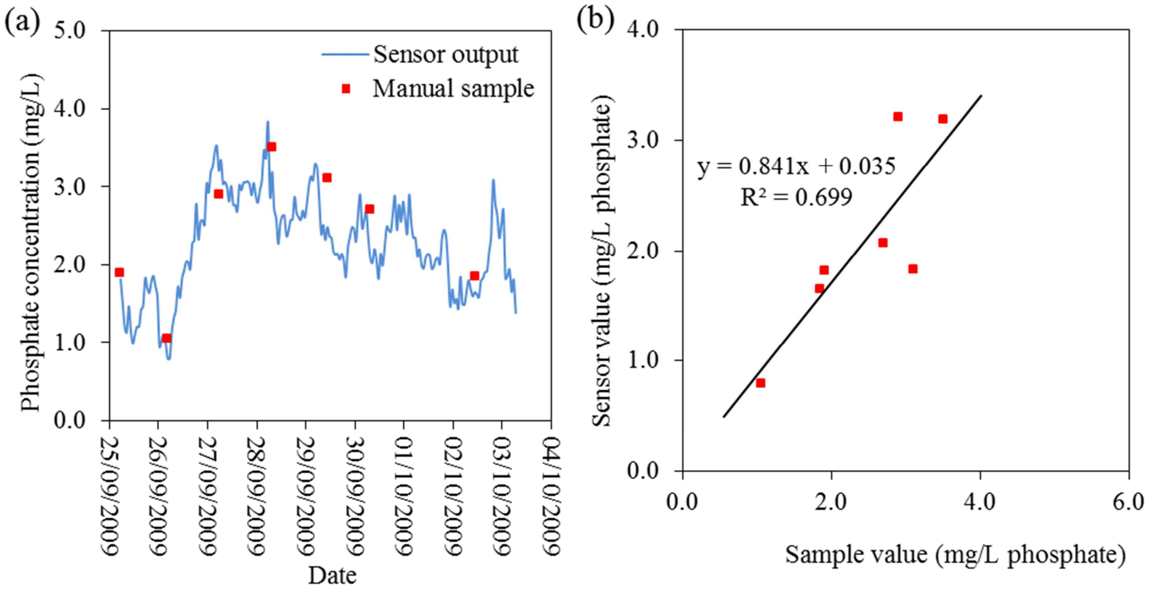

Fig. 8. (a) Phosphate concentrations measured during the first trial period by the sensor and by manual sampling. (b) Sensor output plotted as a function of phosphate concentration as measured by analysis of manually collected samples.

Discrepancies between standard, sampling-based approaches to water quality monitoring and sensor-based approaches clearly represent a barrier to wider uptake of sensor technologies. However, despite these considerations, the data shown in Fig. 8(a) clearly illustrates a key advantage of the deployable monitoring system. The more frequent sensor data showed regular variations in phosphate levels during the trial period which were not captured using daily manual sampling (a relatively high manual sampling rate which is not likely to be practicable in larger-scale monitoring procedures). The sensor-based monitoring is therefore more likely to identify short-term pollution events, as well as providing high-resolution data over long timeframes which can provide valuable information on the long term trends for the analyte of interest.

After 8 days of successful operation, the initial trial was terminated due to clogging of the membrane filter by sediment which had collected around the sampling port. To prevent this problem from recurring, some alterations were made to the sampling port design, and the sensor was deployed close to the original site but at a location with lower susceptibility to sediment build-up on the channel bed. Approximately 480 autonomous measurements were carried out during a second trial period. Figure 9(a) shows that the data obtained during the second trial was broadly similar to that obtained in the first trial period, with phosphate levels generally varying between 2 and 6 mg L^{-1}, and significant peaks and troughs observed on a number of occasions. From 07/11/2009, the data became erratic, resulting in the termination of the trial. Subsequent investigations showed that these erroneous data points were caused by a particulate obstruction in the line delivering reagent to the microfluidic chip, an issue which has since been addressed by careful filtering of all reagent and calibrant solutions prior to use in the sensor. Fig. 9(b) shows that the correlation between sensor output and analysis of manual samples was significantly improved relative to the first trial period, with $R^2 = 0.908$.

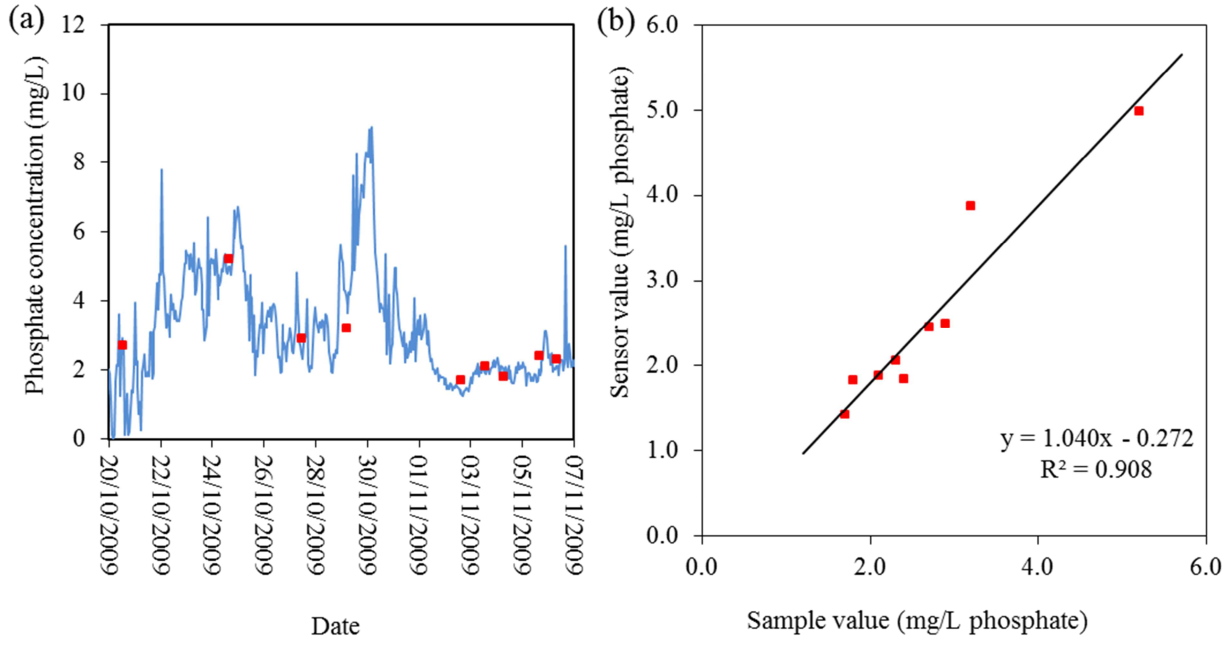

Fig. 9. (a) Phosphate concentrations measured during the second trial period by the sensor and by manual sampling. (b) Sensor output plotted as a function of phosphate concentration as measured by analysis of manually collected samples.

6. Design and *in-situ* testing of second generation phosphate analyser

A substantial redesign of the phosphate analyser was carried out in collaboration with an industry partner (Episensor Ltd., Limerick, Ireland) in order to address some limitations of the system and, in particular, to reduce the component cost of the system to less than €200 per unit while maintaining or improving the analytical performance.

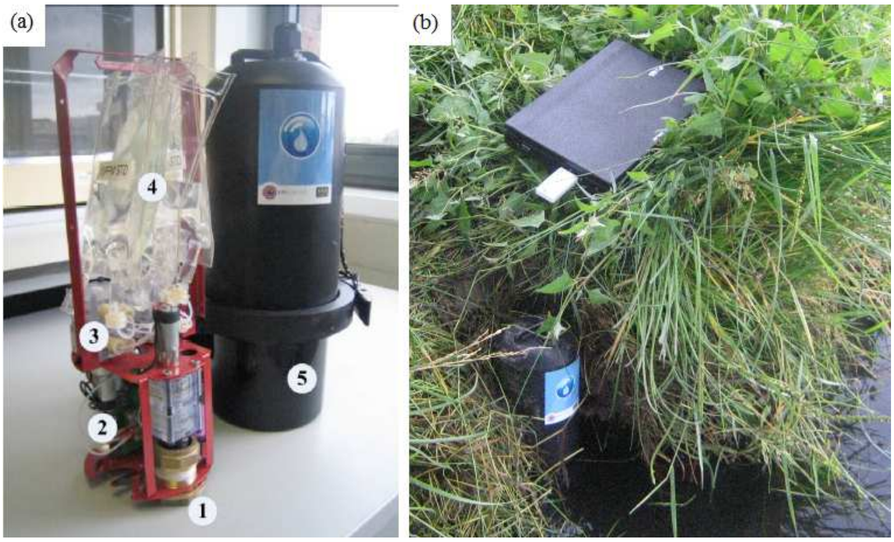

Fig. 10. (a) Second generation phosphate analyser design; (1) Sample inlet; (2) Control board and detection system; (3) Dual channel peristaltic pumps; (4) Reagent bags; (5) IP68 enclosure. (b). The analyser *in-situ* at the Broadmeadow Water test site.

The second generation phosphate analyser is a fully integrated system incorporating fluid handling, microfluidic technology, colorimetric chemical detection, and real time wireless communications in a compact and rugged portable device. The major system components are shown in Figure 10(a). The mode of operation is similar to that of the first generation system. Table I shows some of the significant modifications and improvements implemented in the second generation system. The use of a folded sheet metal frame on which the internal components are mounted, collapsible polypropylene bags for reagent, calibrant and waste storage, and low-cost dual channel peristaltic pumps, have resulted in significant improvements in component cost, compactness and manufacturability. The system also features an improved bubble detection and removal protocol to reduce the impact of bubbles entering or forming within the microfluidic system. The system communicates using short range ZigBee® radio to a remote network gateway, which passes the sensor data via GSM or Wifi to a web-database for remote access by the user. This setup allows the user to not only monitor sensor data in real time but also to remotely change the sensor setup parameters, such as sampling rate.

First Generation System	Second Generation System
Internal Volume: 15L	Internal Volume: 2.3L
System Mass: 12 kg	System Mass: 1.7kg
Battery: 12V Lead Acid	Battery: 3.6V Lithium
Battery Life: 2 months	Battery Life: 12 months
Comms: GSM Modem	Comms: ZigBee Radio
Delayed Reporting	Real time 'sensor to database' reporting

Table I. Comparison of first and second generation phosphate analysers.

An additional change was made to the design of the sensor's sample intake module. This involved the use of commercially available copper-containing fitting to hold the membrane filter. In addition to being cost-effective, the copper content was expected to be of benefit in terms of protecting the membrane filter from biofouling. Copper, copper alloys and copper compounds are widely used as biofouling inhibitors for sensors and other devices deployed in aquatic environments [36,37]. The sensor intake was also located at the bottom of the sensor unit. In addition to being a practical placement for deployment purposes, this had the benefit of shielding the membrane filter from ambient light. Light favours growth of micro-organisms which can promote the formation of biofilms on surfaces. The membrane filter used was a hydrohophilic polyethersulfone membrane designed for biological sample preparation, with low protein binding properties [38]. As attachment of macromolecules (organic and inorganic) and microorganisms represent the initial stages in the biofouling process [36,37], it was hoped that this combination of measures would delay and/or limit biofouling of the filter surface. The redesigned sample intake/filter assembly is shown in Fig. 11.

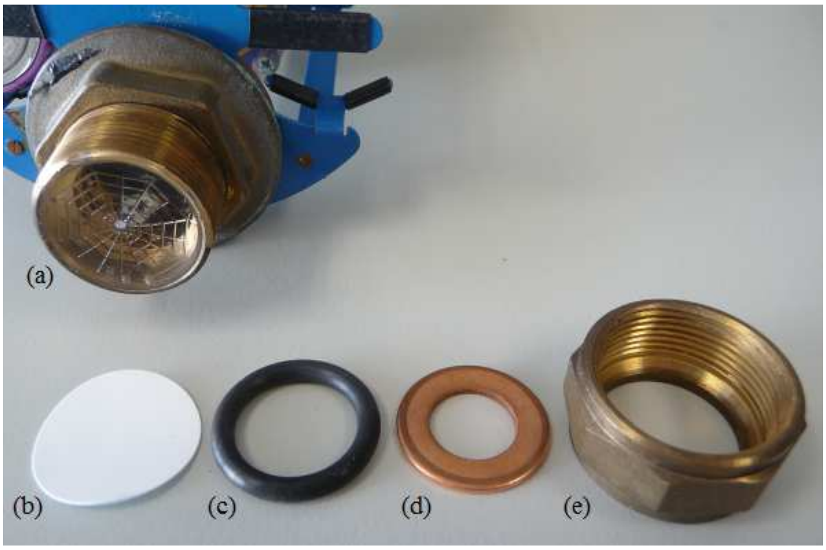

Fig. 11. Components of the sample intake/filter assembly. (a) "spider-web" PMMA filter backing, (b) polyethersulfone membrane filter, (c) O-ring seal, (d) copper washer, (e) brass screw-on fitting.

A "spider-web" pattern is formed on the surface of a PMMA disc by micro-milling. The disc is then fitted into a brass connector where it acts as a backing for the polyethersulfone membrane filter. The spider-web pattern allows a higher proportion of the membrane surface to be used for sample intake, while minimising the dead volume created. The membrane is held in place using an O-ring seal, a copper washer and a brass threaded nut. These copper/brass fittings allow the filter to be easily exchanged/replaced while also offering a degree of protection from biofouling.

Following laboratory validation and calibration, the second-generation analyser was deployed at Broadmeadow Water Estuary, Co. Dublin, Ireland on 04/09/2010. The sensor output over the 62-hour deployment period is shown in Fig. 12(a). The sensor performed 124 autonomous measurements during the trial, and four manual samples were collected for laboratory analysis. These samples were analysed in the laboratory using a Hach-Lange DR890 Portable Colorimeter. Fig. 12(b) shows the excellent correlation (R^2 = 0.9706) between the sensor output and the manual sample phosphate concentrations, a significant improvement over the correlations achieved with the first generation system. The manual sample collected on 06/09/2010 shows the largest deviation from the sensor value. On that occasion, the tidal level at the site did not allow this sample to be taken close to the sensor sample inlet, which may be responsible for the discrepancy observed. All other manual samples were taken directly adjacent to the sensor sample inlet.

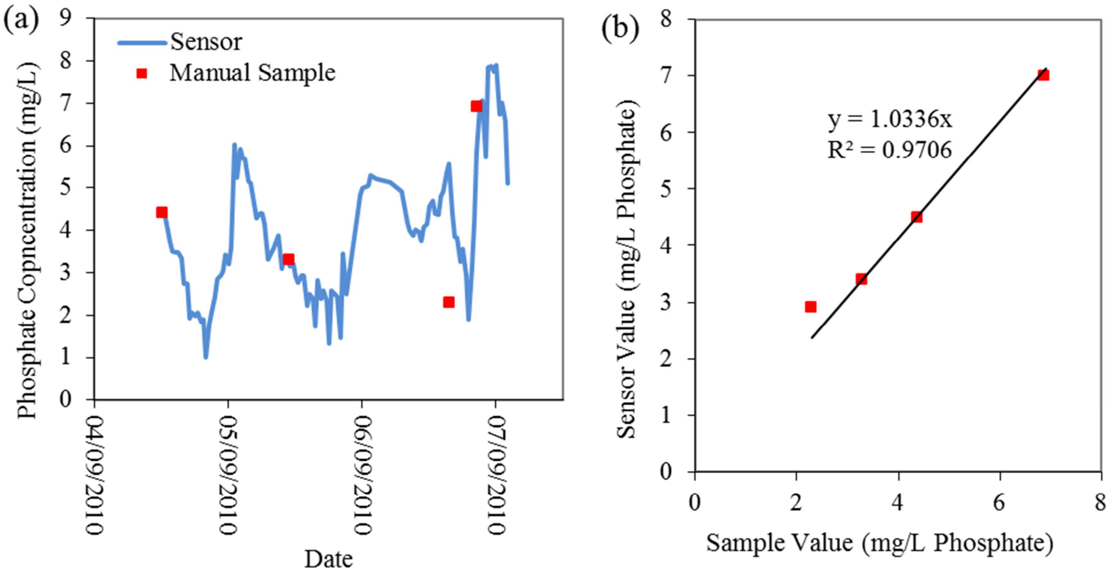

Fig. 12. (a) Phosphate concentrations measured during field trialling of the second generation phosphate analyser at Broadmeadow Water. (b) Sensor output plotted as a function of phosphate concentration as measured by analysis of manually collected samples.

The second generation phosphate analyser has also been used in a wastewater monitoring application. This trial was also carried out at Osberstown Wastewater Treatment Plant, Co. Kildare, Ireland. However, in this case, the analyser was located in the plant’s treated effluent discharge tank, as shown in Fig. 13(a). The sensor module was directly immersed in the effluent, while the communications module was mounted above the water level. This allowed wireless communication using Episensor’s ZigBee® radio system, to a base-station located nearby. An autosampler (Buhler BL 2000, Hach-Lange Ireland Ltd.) was used to collect validation samples at 7-hour intervals, these were stored within the refrigerated compartment of the autosampler, collected periodically, and analysed in the laboratory using the vanadomolybdophosphoric acid method (yellow method) for phosphate detection and a UV-vis spectrophotometer (UV-1600PC Spectrophotometer, VWR International). For sensor validation purposes, this method was preferable to relying on data from the plant’s online phosphorus monitor, as it allowed a more direct comparison between sensor readings and sample values, since (i) the sensor also utilises the yellow method, and (ii) the collected samples were filtered before analysis, so that the sample values represented the dissolved molybdate reactive phosphate fraction only, again in accordance with the actual sensor measurements.

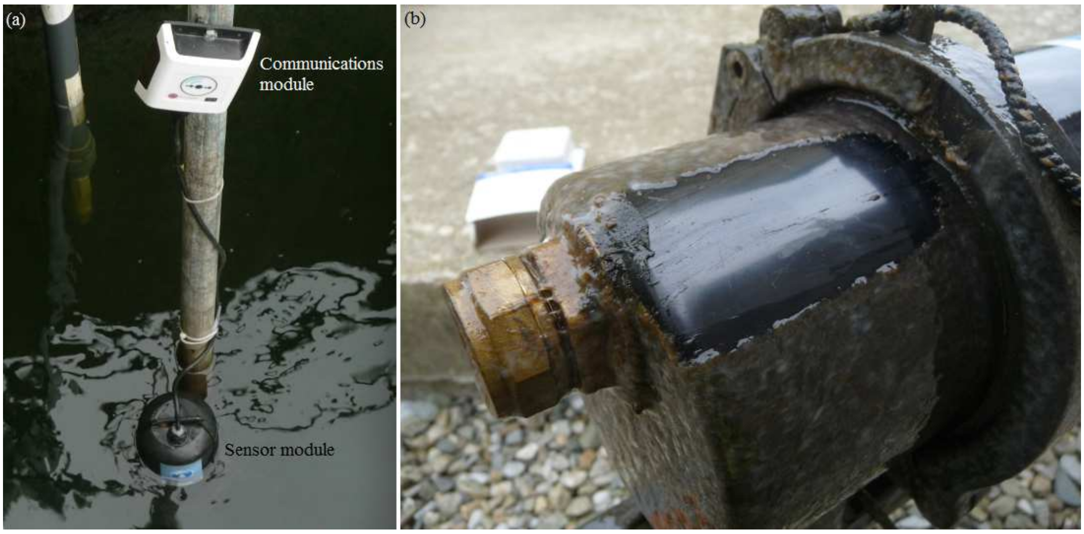

Fig. 13. (a) Second generation phosphate analyser *in-situ* at Osberstown Wastewater Treatment Plant, Co. Kildare, Ireland. (b) Biofouling on the analyser body after approx. 2 weeks immersion in wastewater.

Fouling of the sensor body was a significant feature of this trial. Fig. 13(b) shows the partially cleaned sensor module after removal from the effluent tank after approx. 14 days immersion. A fouling layer approximately 1 mm in thickness was observed. The copper-containing filter housing was noticeably less affected than the sensor body. Fig. 14(a) shows that the membrane filter was also fouled with a thin, adherent layer of material, although there was no discernible effect on the sensor performance.

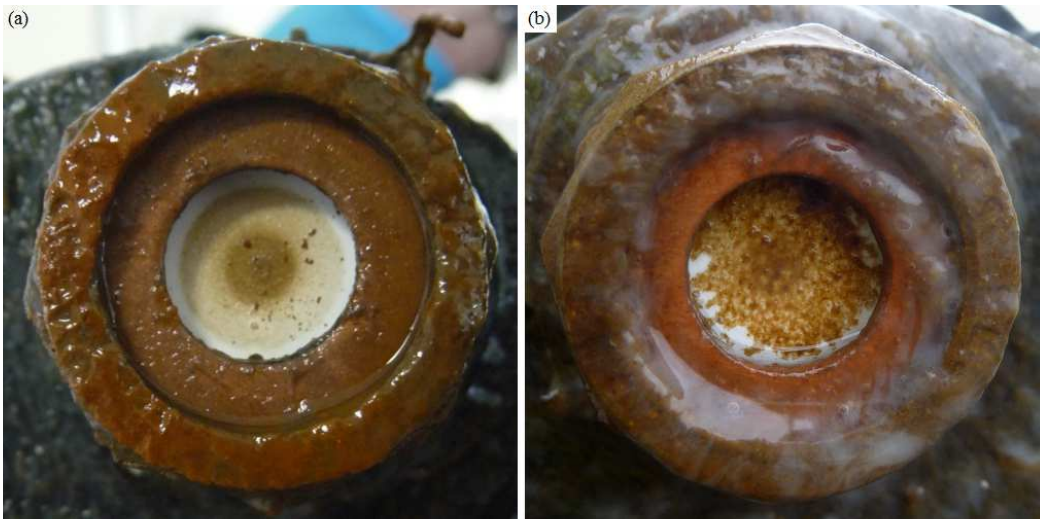

Fig. 14. Fouling of membrane filters and filter housings after approx. 14 days (a) and 10 days (b) immersion in wastewater during a sensor trial at Osberstown Wastewater Treatment Plant.

The filter was replaced and the system was replaced in the effluent tank. At the end of the trial (after a further 10 days, approximately) the membrane and housing were again examined. At this point the filter holder was heavily fouled as shown in Fig. 14(b). A layer of fouling material was again in evidence on the membrane filter itself. However, in this case the material was found to be only loosely adherent to the filter surface, and was readily removed with a deionized water wash bottle. This qualitative difference between the types of fouling material on the two filters highlights the variability of fouling which can occur over a relatively short period of time, presumably due to changes in sample composition and prevailing weather conditions. While no specific effect of fouling on sensor performance was observed in this trial, the rapid build-up of material suggests this could be an issue in longer-term deployments in wastewater and in other sample matrices. This is especially relevant in applications such as marine waters or oligotrophic freshwaters where very low levels of phosphate (or other nutrients) need to be detected. In such scenarios, local uptake of nutrients by biofilm on sensor or filter surfaces could significantly impact on the measured value.

Fig. 15 shows the data collected the sensor during the trial, as well as the results of samples collected by auto-sampler and analysed in the laboratory. The sensor and sample values are generally in good agreement. The elevated phosphate levels during the first 5 days of the trial were due to the plant's phosphorus removal system (based on dosing with ferric chloride) being out of operation. After this system was restored to operation on 15/05/2011, the phosphate values quickly returned to normal levels, generally between 0.5 and 3.0 mg/L PO_4^{3-}. Sensor data was not collected during the period 22/05/2011–25/05/2011 due to maintenance works at the plant during this period, resulting in the water level in the effluent tank being below the sensor's sample intake port. As soon as the water level returned to normal and sample was again accessible to the sensor, the readings automatically recommenced and once again are in good agreement with validation sample values for the duration of the trial. This is encouraging as it demonstrates the sensor's robust ability to cope with an interruption of this nature and to clear the air which would have been drawn into the microfluidic system while the sample was inaccessible.

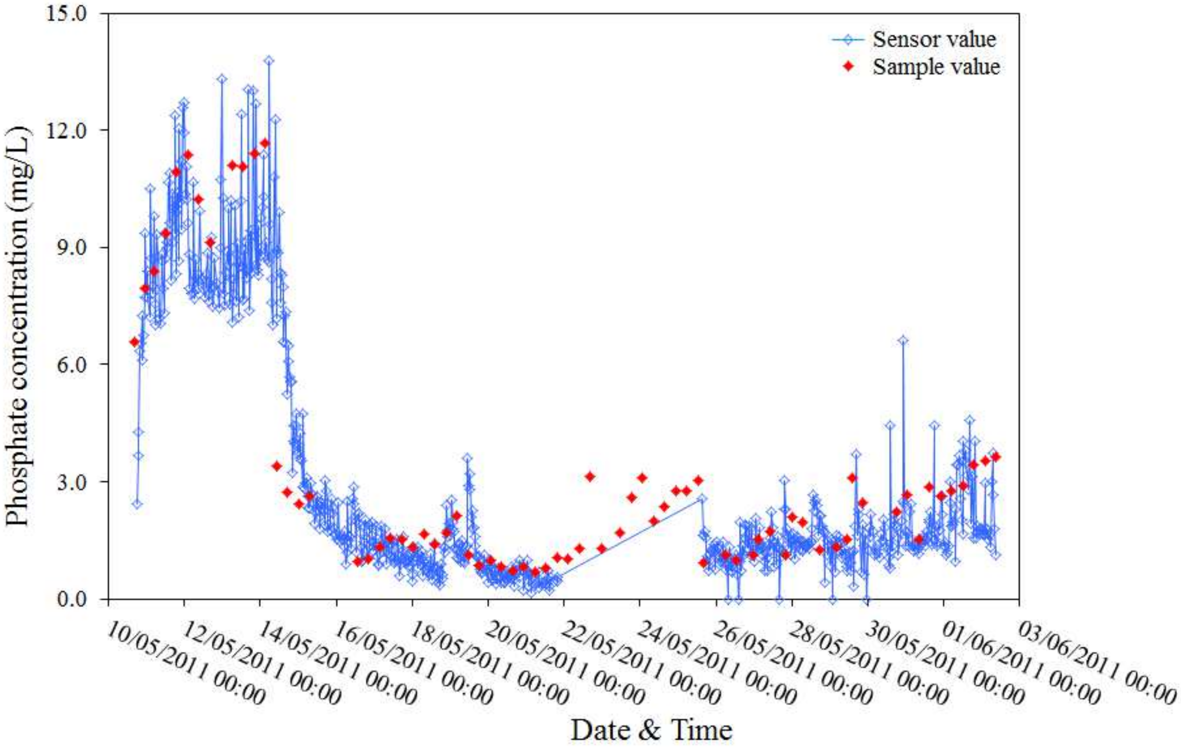

Fig. 15. *In-situ* sensor data (sensor value) and validation data (sample value) from samples collected using an auto-sampler during a trial of the second generation phosphate sensor at Osberstown Wastewater Treatment Plant.

7. Current status and further developments of the system

The data presented here shows that the microfluidic analyser which has been developed represents a viable, low-cost and reliable method for long-term monitoring of phosphate across a range of applications including wastewater and surface water monitoring. While the current limit of detection of approximately 0.2 mg L^{-1} PO_4^{3-} represents a limitation on the applicability of the system, it can be expected to find significant applications in the monitoring of in-process and discharged effluent from municipal wastewater treatment plants, industry and other point sources of phosphate, as well as in the monitoring of surface waters known to be impacted by phosphate pollution. Moreover, Jordan and co-workers [39,40] have shown the importance of high temporal resolution monitoring for the detection of short-term, high-phosphorus concentration inputs to catchments that typically have low background levels of phosphorus. Detection of this kind of short-lived pollution event can be crucial to understanding the overall nutrient dynamics within a water body, and can only be reliably achieved using *in-situ* analytical devices.

Current applications notwithstanding, on-going efforts are aimed at improving the analytical performance of the phosphate sensor, particularly the limit of detection, so as to broaden the applicability and maximise the marketability of the system. Additional long-term field deployments are also planned, with the objective of validating the long-term performance of the analyser in different applications, and examining the effect of biofouling during these long-term deployments. Biofouling potential is likely to vary significantly across different applications (e.g. wastewater, marine, freshwater), and between different locations in the same water system, and is also dependent on weather conditions and time of year. In general, however, biofouling is likely to represent a practical limitation to the (maintenance-free) deployable lifetime of this sensor and similar systems. Therefore it is essential that developers of water monitoring technology are familiar with the range of established and emerging [36,37,41-44] approaches to the prevention or limitation of biofouling, and work collaboratively with researchers engaged in this area, which is a key to achieving the long deployable lifetimes which will ultimately facilitate the broader uptake of *in-situ* sensing technologies.

The capabilities of the microfluidic analytical platform are also being expanded to allow detection and monitoring of a range of additional important water quality parameters. This work is being progressed via a number of distinct projects, and the range of parameters being targeted includes nitrate, ammonia, nitrite, pH chemical oxygen demand (COD), and metals such as cadmium, mercury and lead. Colorimetric techniques for the detection of the various analytes are being assessed or developed, and optimised for application in microfluidic analytical devices, with the aim of developing a suite of single- or multi-parameter sensing systems which can be applied to the monitoring of water quality across a range of applications.

References

[1]. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

[2]. Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water collection and treatment.

[3]. Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution by nitrates from agricultural sources.

[4]. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption

[5]. Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006, on the protection of groundwater against pollution and deterioration.

[6]. T. S. Moore, *et al.* “Marine chemical technology and sensors for marine waters: potentials and limits”, *Annual Review of Marine Science*, vol. 1, pp. 91-115, 2009.

[7]. M. Dzakpasu, *et al.* “Nitrogen removal in an integrated constructed wetland treating domestic wastewater”, *Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* Vol. 46, pp. 742-750, 2011.

[8]. A. Manz *et al.*, “Miniaturised total chemical analysis systems, a novel concept for chemical sensing”, *Sensors and Actuators B: Chemical* vol. B1, pp. 244-248, 1990.

[9]. R. Niessner, “Measuring the invisible”, *Analytical Chemistry*, vol. 82, pp. 7863-7863, 2010.

[10]. Allan, I.J. *et al.* “A toolbox for biological and chemical monitoring requirements for the European Union's Water Framework Directive”, *Talanta*, vol. 69, pp. 302-322, 2006.

[11]. Diamond, D. *et al.* “Integration of analytical measurements and wireless communications - current issues and future strategies”, *Talanta*, vol. 75, pp. 606-612, 2008.

[12]. Gardolinski, P.C.F.C. et al. “Miniature flow injection analyser for laboratory, shipboard and in situ monitoring of nitrate in estuarine and coastal waters” *Talanta*, vol. 58, 1015-1027, 2002.

[13]. G.N. Doku and S.J. Haswell, “Further studies into the development of a micro-FIA (mu FIA) system based on electroosmotic flow for the determination of phosphate as orthophosphate” *Anal. Chim. Acta*, vol. 382, pp. 1-10, 1999.

[14]. G.M. Greenway *et al.* “Characterisation of a micro-total analytical system for the determination of nitrite with spectrophotometric detection”, *Anal. Chim. Acta*, vol. 387, pp. 1-10, 1999.

[15]. P.H. Petsul *et al.* “The development of an on-chip micro-flow injection analysis of nitrate with a cadmium reductor” *Anal. Chim. Acta*, vol. 428, pp. 155-161, 2001.

[16]. A. Daridon *et al.* "Chemical Sensing using an Integrated uFluidic System based on Colorimetrics: A Comparative Kinetic Study of the Bertholet Reaction for Ammonia Determination in Microfluidic and Spectrophotometric Systems", *Sens. Actuators B,* vol. 76, pp. 235-243, 2001.

[17]. F. Azzaro and M. Galletta, "Automatic colorimetric analyzer prototype for high frequency measurement of nutrients in seawater", *Marine Chemistry*, vol. 99, pp. 191-198, 2006.

[18]. P. Moscetta and I. Thompson, "The new in-situ chemical probes and the water quality monitoring platform" *Environmental Risk Management Tools for Water Quality Monitoring*, Southampton (UK), 30 March 2009.

[19]. Alliance for Coastal Technologies (2008) *Performance Demonstration Statement for the American Ecotech NUT 1000*,
Available: http://www.act-us.info/Download/Evaluations/Nutrient//AmerEco/

[20]. Alliance for Coastal Technologies (2008) *Performance Demonstration Statement for the WET Labs Cycle-P Nutrient Analyzer* (2008)
Available: http://www.act-us.info/Download/Evaluations/Nutrient/WETLabs/

[21]. Alliance for Coastal Technologies, *Performance Demonstration Statement for the YSI 9600 Nitrate Monitor* (2007)
Available: http://www.act-us.info/Download/Evaluations/Nutrient/YSI/

[22]. R. Vuillemin *et al.* "CHEMINI: A new in situ chemical miniaturized analyzer" *Deep Sea Research Part I: Oceanographic Research Papers*, vol. 56, pp. 1391-1399, 2009.

[23]. M.D. Patey, *et al*. "Determination of nitrate and phosphate in seawater at nanomolar concentrations", *Trends in Analytical Chemistry,* vol. 27, pp.169-182, 2008.

[24]. V.J. Sieben *et al.* "Microfluidic colorimetric chemical analysis system: application to nitrite detection" *Analytical Methods*, vol. 2, pp. 484-491, 2010.

[25]. Abi Kaed Bey *et al.* "A high-resolution analyser for the measurement of ammonium in oligotrophic seawater" *Ocean Dynamics*, vol. 61, pp. 1555-1565, 2011.

[26]. C.F.A. Floquet *et al.* "Nanomolar detection with high sensitivity microfluidic absorption cells manufactured in tinted PMMA for chemical analysis" *Talanta*, vol. 84, pp. 235-239, 2011.

[27]. A.D. Beaton et al. "Lab-on-chip measurement of nitrate and nitrite for in situ analysis of natural waters.", *Environmental Science & Technology*, vol. 46, pp. 9548-9556, 2012.

[28]. M. Sequeira and D. Diamond, "Progress in the realisation of an autonomous environmental monitoring device for ammonia" *Trends Anal. Chem*. vol. 21, 816-827, 2002.

[29]. M. Bowden *et al.* "Analysis of river water samples utilising a prototype industrial sensing system for phosphorus based on micro-system technology." *J. Environ. Monit*. vol. 4, pp. 767-771, 2002.

[30]. M. Bowden *et al.* "A prototype industrial sensing system for phosphorus based on micro system technology" *Analyst*, vol. 127, pp. 1-4, 2002.

[31]. C. Slater et al. "Autonomous field-deployable device for the measurement of phosphate in natural water", *Proc. SPIE 6755, Advanced Environmental, Chemical, and Biological Sensing Technologies V,* 67550L, pp. 67550L1-67550L8, 2007.

[32]. C. Slater *et al.* "Validation of a fully autonomous phosphate analyser based on a microfluidic lab-on-a-chip" *Water Sci Technol*, vol. 61, pp. 1811-1818, 2010.

[33]. Environmental Protection Agency, Ireland (2005) *Water Quality in Ireland, 2001-2003*, Available: http://www.epa.ie/downloads/pubs/water/waterqua/epa_water_quality_2001-3.pdf

[34]. C. Neal, et al. "Phosphate measurement in natural waters: two examples of analytical problems associated with silica interference using phosphomolybdic acid methodologies", *Sci. Total Environ*. vol. 251/252, 511-522, 2000.

[35]. Hach Company 1999, *DR/890 Datalogging Colorimeter Handbook*, pp. 523-528.

[36]. T. Sullivan, and F. Regan, "Biomimetic design for the development of novel sustainable antifouling methods for ocean monitoring technology" *J. Ocean Technol*. 6, pp. 41-54, 2011.

[37]. L. Delauney *et al*. "Biofouling protection for marine environmental sensors" *Ocean Sci*., vol. 6, pp. 503-511, 2010.

[38]. PALL Corporation, *Polyethersulfone Membrane (Hydrophilic)*, http://www.pall.com/main/OEM-Materials-and-Devices/Product.page?id=3949

[39]. P. Jordan, et al. “High-resolution phosphorus transfers at the catchment scale: the hidden importance of non-storm transfers.” *Hydrol. Earth Syst. Sci*. vol. 9, pp. 685-691, 2005.

[40]. P. Jordan, *et al*. “Characterising phosphorus transfers in rural catchments using a continuous bank-side analyser” Hydrol. Earth Syst. Sci. vol. 11, pp. 372-381, 2007.

[41]. J. Chapman *et al*. “Phthalate doped PVC membranes for the inhibition of fouling” *J. Membr. Sci*., vol. 365, pp. 180-187, 2010.

[42]. J. Chapman *et al*. “Period four metal nanoparticles on the inhibition of biofouling” *Colloids and Surfaces B: Biointerfaces*, vol. 78, pp. 208-216, 2010.

[43]. J. Chapman (2011) *The development of novel antifouling materials: A multi-disciplinary approach*. PhD thesis, Dublin City University.

[44]. T. Sullivan, (2012) *Investigation of biomimetic and engineered surface textures for biofouling reduction and prevention on environmental sensing platforms*. PhD thesis, Dublin City University.

[45]. J. Cleary *et al.* “Analysis of phosphate in wastewater using an autonomous microfluidics-based analyser” *World Academy of Science, Engineering and Technology,* vol. 28, pp. 196-199, 2009.