# Real-time in situ monitoring nutrient fate and hypoxia occurrence in natural water sources

## Basic Information

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## Publications

1. Xu, Zhiheng, Qiuchen Dong, Brunah Otieno, Yucheng Liu, Isaiah Williams, Dingyi Cai, Yan Li, Yu Lei, Baikun Li. (in review). Milli-electrode array (MEA) for real-time in situ profiling of water quality. Environmental Science &Technology.
2. Xu, Zhiheng, Qiuchen Dong, Brunah Otieno, Yucheng Liu, Isaiah Williams, Dingyi Cai, Yan Li, Yu Lei, Baikun Li. (in preparation) Modeling water/sediment physio-chemical fates based on MEA profiles.
3. Xu, Zhiheng Milli-electrode array (MEA) for real-time in situ water quality monitoring. AEESP Annual Conference June 2015. Yale University (New Haven, CT).
4. Xu, Zhiheng. Fabrication of Milli-electrode array (MEA). New England Graduate Student Environmental Engineering Conference Sept. 2015. University of Massachusetts (Amherst, MA).

**Proposal Title.** Real-time *in situ* monitoring nutrient fate and hypoxia occurrence in natural water sources

**Introduction/Research Objective**

Water connects physical, geochemical, biological, and ecological processes occurring on the Earth's surface and in the sediment, and comprehensively links the natural environment with human activities at multiple scales of space and time ranging from small pond to open ocean. Steady modification of coastal watersheds through deforestation and urban development, dense population, and ignorant discharge/dumping of wastes and non-point pollutants have caused severe eutrophication and hypoxia in the large estuaries of in the State of Connecticut and Long Island Sound (LIS).  Achieving "*High Water Quality*" and managing "*Fate and Transport of Contaminants in Air, Water and Soils*" is a prior initiative for Connecticut, and requires the innovative real-time *in situ* sensing technology.  Moreover, understanding the biogeochemical cycle in natural water resources is critical for us to protect environment and manage water availability and biodiversity. Before any treatment process is designed, policy and regulation is developed, and protection and restoration strategy is executed, a comprehensive picture of ecosystems at physiochemical aspects should be acquired.

Currently, inorganic/organic contaminants (e.g. nitrogen, phosphate, organic carbon, and heavy metals) in water and sediment are measured using chromatography, atomic absorption (AA), induced coupled plasma (ICP), and fluorimetry. These techniques normally require samples to be collected and sent to the off-site laboratory for analysis.  Problems were often encountered, such as pressure/temperature/oxygen changes for sediment samples and hydrothermal samples. These may result in systematic errors, and ultimately lead to misinterpretation of the results observed. With the development of microelectrodes (MEs), microprofiling becomes possible. MEs are micro-scale needle-shaped electrodes capable of penetrating into environmental samples (e.g. biofilms, activated sludge, and sediment) at different depths with the control of micromanipulators. The PI Li developed a ME setup to measure activated sludge flocs in wastewater treatment plants (Figure 1a).  MEs technology can obtain the chemical micro-profiles without destroying samples. However, the needle-shaped MEs have three major problems hindering the real time measurement.  **First,** most MEs can only measure a single parameter (e.g. oxygen, nitrogen, and pH) and only obtain one data point during each penetration into a sample, which makes it impossible for real-time measuring water-sediment environment. **Second,** these MEs require tedious fabrication procedures with low success rate and reproducibility**.**  MEs are fragile and can not be reusable for multiple measurements. **Third,** the whole ME setup is complicated. The movement of MEs is controlled by a micromanipulator (Figure 1a and 1b), which makes it impossible to deploy for *in situ* measurement at harsh sites, such as deep-sea sediment.
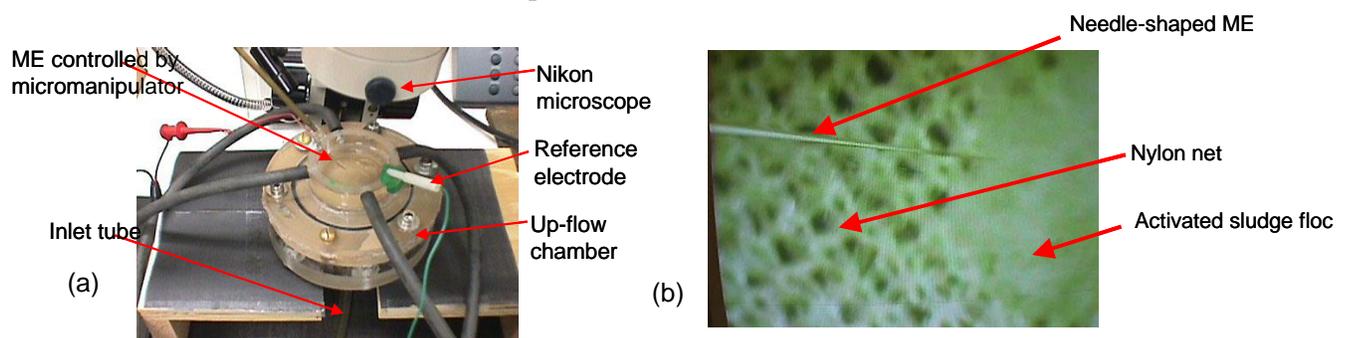


**Figure 1.** The experimental setup of the needle-shape microelectrode (ME) measurement of activated sludge flocs.  (a) The whole ME system setup.  (b) Penetration of a ME into activated sludge flocs .
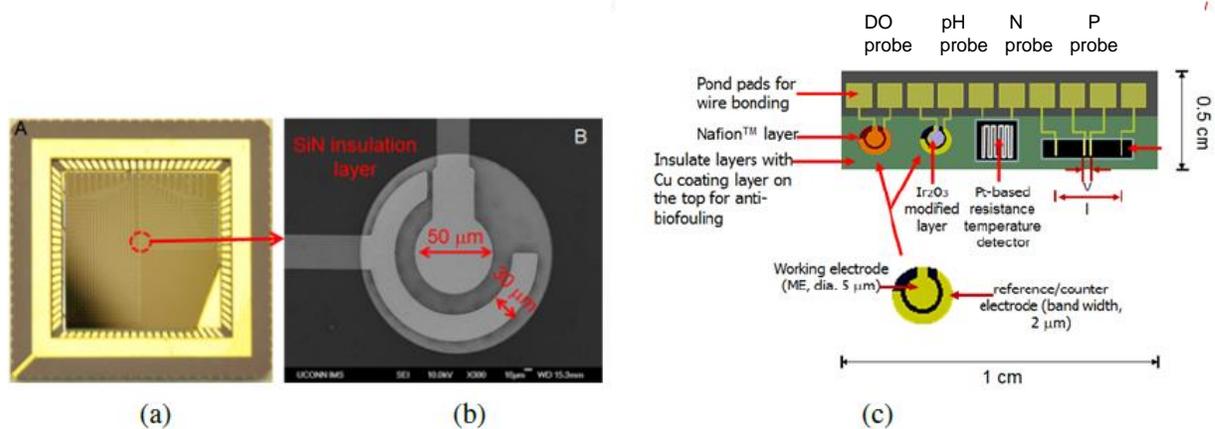
**Figure 2.** (a) Optical image of a microfabricated MEC device. (b) The SEM picture of one pair of microelectrodes (ME). (c) Schematic of the array of four types of MEs (not in scale).

To solve the ME problems for real-time *in situ* long-term monitoring, the research team led by the PIs has already fabricated a new generation micro-measurement device, all-in-one microelectrode chip (MEC) in a *NSF supported project (CBET 1336425)*. By precisely patterning multiple (e.g. 20 or 50) Cr-Au microelectrodes (MEs) on a single silicon wafer, a MEC is capable of measuring multiple parameters along the depth of samples (Figure 2a). The silicon dioxide layer is thermally grown on the silicon wafers for insulation. Each ME consists of a working electrode (diameter: 50 µm) and a circular strip reference/counter electrode (width: 30 µ m) surrounding the working electrode (Figure 2b). Compared to the existing sensors in which reference/counter electrodes are separated far away with high-power consumption, the unique MEC pattern is more accurate and sensitive, and minimizes the power consumption. The MEC has four key advantages over traditional MEs: easy fabrication, sturdy configuration with long lifetime, simple measurement procedure without the need of micromanipulator, and measuring/profiling multiple parameters, which provides an excellent sensing platform to characterize the migration fate of contaminants in real-time format. The PIs have conducted oxygen detection in phosphate buffer solution and obtained high precision.

The PIs have developed a multi-module MEC for simultaneous measurement of four critical parameters related with water quality (dissolved oxygen (DO), pH, nitrogen, and phosphorous). Briefly, the MEs (each ME:20-nm Cr/100-nm Pt) were modified for specific parameter. An array of these four types of MEs were micro-fabricated and integrated on the same silicon chip (as a MEC) to minimize the sensor energy consumption for elongated operation time (Figure 2c). The MEs specific for each parameter were laid out in horizontal direction and extended to the vertical direction on the MEC. Nitrate, phosphate, and pH will be measured potentiometrically, and DO will be measured amperometrically. A copper (Cu) layer will be coated on the MEC surface as the anti-fouling solution for long-term monitoring [17]. The MECs overcome the problems of existing macro-electrodes and micro-electrodes, and can achieve a simultaneous measurement of multiple parameters at different depths of a given aquatic system. The PIs have developed a water-proof cartridge (1.5×1.5 cm) to hold the MEC (Figure 3) and connected with a data logging system through an external circuit. Each MEC chip has at least 50 MEs along the horizontal and vertical directions, which can be easily inserted into the test site (e.g. bulk water, sediment, and soil) to monitor multiple parameters at multiple depths.

The MEC is an excellent measurement tool for multiple parameters. Successful on-site application of this novel MEC technology can give insight to spatial physiochemical distributions in microenvironment, and will yield profound significance for modeling and designing a site of interest. This project targets the rapid sensing and characterization of natural water resources using MECs. The profiles will be integrated with kinetic models to elucidate the occurrence of eutrophication and hypoxia.
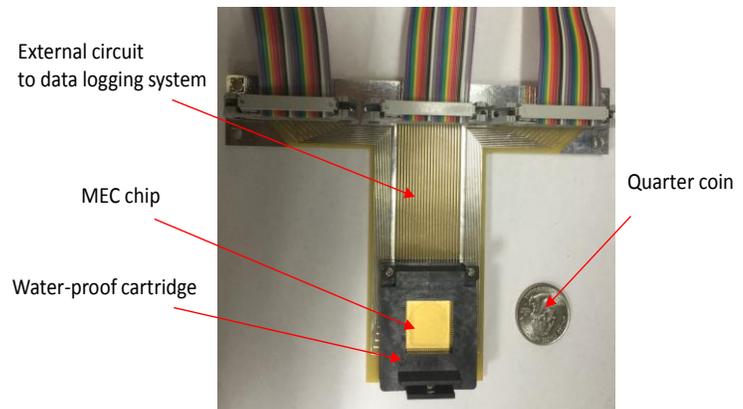


**Figure 3.** Water-proof MEC cartridge with each chip (1.5cm×1.5cm) holding at least 50 MEs in the horizontal and vertical direction.

This project features many innovations that have the potential to revolutionize the *in situ* real-time measurement of eutrophication and hypoxia in the State of Connecticut and the LIS. **First**, the lab tests and the field tests will testify the feasibility and accuracy of MECs for on-site monitoring, and make the *in situ* real-time profiling multiple parameters at multiple depths possible. *No any existing sensing technology has this unique feature.* The outcomes will lay a solid foundation for protecting, preserving and restoring a broad stratum of water-related systems (e.g. water/sediment, deep sea, boat hull, water supply networks, and water/wastewater treatment facilities) and solid-related systems (e.g. bioremediation sites and Brownfields). **Second**, by obtaining the real-time *in situ* profiles of nutrient and oxygen/pH in natural water resources, this study will quantify the dynamic variations and extrapolate nutrient flow patterns. This information will enable researchers and engineers to elucidate the contaminant fate across broad physical expanses and times and design efficient processes to minimize pollutant impacts. **Third**, the project will transform the way in which we observe the vulnerable aquatic environments and the fragile ecosystems they host, and provide the technical guidelines for regulators and policy makers to execute timely and efficient strategies. By developing high spatial/temporal resolution for substance migration, the project will have a significant contribution to nutrient reduction and hypoxia solution in the State of Connecticut, and bring us closer to the ultimate goal of U.S. long-term environmental sustainability.

The success of this multidisciplinary project will bridge the gap between state-of-the-art electrochemical sensing technology and rapid characterization of dynamic aquatic systems. The project is strongly correlated with the Connecticut Water Quality Protection and Preservation Initiative of the Connecticut Department of Energy Environmental Protection (CTDEEP), the Mission "resolve state and regional water related problems" of the Connecticut Institute of Water Resources (CTIWR), and Water Initiatives and Environmental Cleanup Initiatives at the University of Connecticut (UConn). Moreover, this project will strengthen the broad collaboration that the PIs have established at Departments of Electrical Engineering, Material Science, Chemistry, Microbiology, Marine Science, Nanoelectronics Center, Center for Environmental Science and Engineering (CESE), and Center for Clear Energy Engineering (C2E2). Several funding agencies (EPA, DOE, and DOD) have shown strong interests in the PIs' vision for the application of MECs in engineered systems and natural resources. These

program directors have acknowledged that obtaining an accurate physicochemical spatial stratification in a given ecosystem is the very first step to develop effective protection and restoration strategy. The lab-scale and field tests of MECs in this project will build a foundation to apply these external funds.

<u>Hypoxia is closely related with nutrient contents, and nutrient reduction could be reflected by the extents of hypoxia in natural water ecosystems.</u> <u>The objective of this multidisciplinary proposal </u> is to achieve the real-time *in situ* profile of nutrient fate and oxygen content using the MECs that the PIs have developed, and apply models to predict eutrohphication and hypoxia occurrence. <u>The project was proposed in three steps</u>: (1) To apply the micromanipulator-free and individually addressable MECs in the lab-scale system and measure hypoxia occurrence and water quality, (2) To apply the MECs to two real water resources (Swan Lake and Fenton River), determine the nutrient transfer along the depths of water bodies at different seasons, and correlate hypoxia and nutrient contents, and (3) To predict the long-term migration fate of nutrients and hypoxia at these two water bodies using a mass transfer model developed. *In this project, the interface of water and sediment will be selected as the target, which represents a broad spectrum of natural environments and biodegradation sites. The contaminants will be represented by nitrogen and phosphorous, which have caused severe eutrophication in Connecticut water resources.* <u>The </u>*rationale*<u> of this research </u> lies in the acquisition of the profiles of diverse parameters at multiple depths using MECs, so that the occurrence of eutrophication and hypoxia can be characterized as a whole set of picture and nutrient loads can be real-time *in situ* monitored. This project holds great promise to enhance our understanding of migration fates of contaminants in natural water resources.

**Methods/ Procedures/Progress.**

The variation of nutrient and oxygen concentrations in water resources was first examined in a lab-scale simulated aquatic system. The water and sediment was taken from the Swan Lake on the University of Connecticut (UConn) campus, and put in a continuous-flow tank (volume: 20 L) in the lab (Figure 4). The Swan Lake intakes the wastewaters from the UConn Wastewater Treatment Plant (UConn WWTP), which treats diverse wastewaters (e.g. office, dormitory, cafeteria, and labs) and contains diverse inorganic/organic compounds. <u>Two critical environmental parameters</u> (organic concentration and nitrogen concentration) have been tested. Chemical oxygen demand (COD), a commonly used parameter was selected to indicate organic contaminant concentrations on site. COD is related with bacterial activities and groundwater quality, and its variation will affect the removal of nitrogen and phosphate in water/wastewater systems and oxygen levels in water resources. High total nitrogen concentration (TN, organic nitrogen and inorganic nitrogen) of water bodies directly causes eutrophication and affects oxygen levels, pH, and microbial communities. These two variables were tested individually to simulate waste
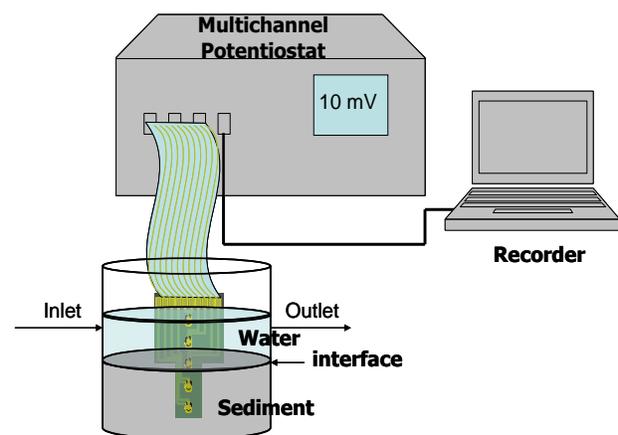


**Figure 4.** Schematic of the lab-scale setup for the profiling in the interface of water and sediment with a "T"-shaped MEC (not in scale).

discharge in natural water resources.

The system was operated at the COD of 50-100 mg/L, TN concentration of 15 mg/L, flow rate of 5L/hr, and temperature of 24 °C as the standard stable status. Four types of MECs (DO, pH, N, and P) developed in the PI's groups (as shown in Figure 2c) was inserted into the tank. To easily identify the position of water/sediment interface, the MEC will be designed as the "T"-shape, with the water/sediment interface being matched with the T"-shaped interface position marker (Figure 4). Actually, this is one of the unique features of the MEC, which avoids the usage of complicated micromanipulators to determine the MEs' positions on the monitoring site. Four profiles (DO, pH, N, and P) measured by each ME were collected real-time using a multichannel potentiostat and a data logging system, and saved in an MS EXCEL file (Figure 4). *All these facilities are available at the PIs' labs.* The main reason for conducting the tests in the lab-scale system first rather than in field is that a well controlled lab environment is crucial to verify the applicability of MECs.

After the lab-scale system is operated at the standard status for 3-4 weeks and the MECs reach the stable readings for all profiles, two variables (COD concentration and TN concentration) was introduced to the system at two levels (high and low) separately to simulate low concentration at raining season and high concentration after waste discharge: COD concentrations (20 and 500 mg/L) and TN concentration (2 and 40 mg/L). Different dosages of sodium acetate and ammonium chloride were individually added in the influent to achieve high/low COD and N concentrations. Each condition was examined for 3 weeks, during which the profiles of nutrient (N and P), oxygen, and pH will be simultaneously obtained in the water and sediment phases. The reproducibility of the MEC measurements was verified by conducting at last triplicate tests (each test duration: 3-4 days) under each condition.

*Calibration and validation of MECs.* The calibration and validation of MECs was performed before each measurement in the field tests. MECs were calibrated with the commercialized microelectrodes and macroelectrodes at different concentrations of dissolved oxygen, pH, $NO_3^-$, and $PO_4^{3-}$ solutions. The accuracy and reliability of MECs was checked.

**Results/Significance**

The most significant result of the project was the 2D MEC development capable of monitoring multiple water-quality related parameters with high spatial and temporal resolution. Five types of MEC sensors (redox potential (ORP), pH, temperature, conductivity, and ammonium $NH_4^+$) specifically for water quality have been developed at the PIs' labs by precisely patterning multiple mm-sized electrodes using an inkjet printer (**Figure 5a**). Multiple rows vertically compose a MEC matrix capable of simultaneously measuring multiple parameters along depth (e.g. 4 cm (width) × 30 cm (depth) hosting 60 electrodes) (**Figure 5b**). Each type electrode were modified for specific parameter using the reported procedures, with a bond pad on the top of the film for wire bonding, allowing the easy connection to external circuit (**Figure 6**). For ORP, pH and $NH_4^+$ sensors, three-electrode systems include same pattern gold-based working and counter electrodes, and silver-based reference electrodes (**Figure 6**). Then, the working electrode of pH sensor is modified through the deposition of $Ir_2O_3$[74,75], and the working electrode of $NH_4^+$ sensor is modified with a coating of polyaniline. Temperature sensor is made by zip-shape gold-based resistance and conductivity sensor consists of four gold-based electrodes as a capacitor **(Figure 6)**.

The unique designed MECs are more accurate and sensitive, since the electrochemical signals can be immediately and fully collected by the adjacent counter electrode (**Figure 6**). This is very different from the existing electrodes which need a separated reference/counter electrode. The ink-jet printed MEA sensors are low cost ($0.2 per sensor, mainly gold/silver ink cost) and easy to mass fabricate. The precise alignment of each electrode on the film makes the MEC individually addressable, which substantially eases the *in situ* measurement on the field. Electrochemical signals of MECs are recorded using a multiple-channel data-logging system. Specifically, $NH_4^+$ concentration is measured using current, pH and ORP using potential, conductivity using capacitance, and temperature using resistance.

## MEC Calibration and Response to Variation of Parameters.

Each type sensors of MEAs are calibrated to establish an accurate correlation between electrochemical signals and the targeted parameters. The calibration has been conducted in water solutions that well represent typical water quality, with ORP of -350--+300 mV, pH of 2-10, temperature of 10-50°C, conductivity (indicating dissolved solid content) of 1500-3000 µS/cm and $NH_4^+$ 20-100 mg/L. Each type sensor showed high accuracy between electrochemical signals and the targeted parameters with $R^2$ values higher than 99% (**Figure 7a**). Besides calibration, a shock test was conducted by rapidly changing water temperature and conductivity to simulate water system malfunction (e.g. temperature fluctuation and fatty acid accumulation in AD systems). MEA sensors showed the real-time signal change with the shock (**Figure 7b.** *only showing conductivity and temperature response due to the space limit*), indicating an excellent capacity of capturing the shock of impaired water quality. It should be noted that mm-sized electrodes on the MEC are not only limited to these five parameters. Each electrode can be easily modified using surface coating and electrochemical deposition to monitor wide range of parameters (e.g. toxic metal ions, pesticide, salt, and nutrients, etc.).
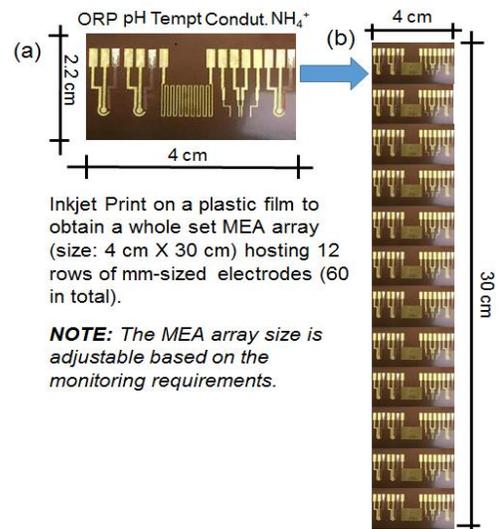


**Figure 5.** MEC ink-jet-printed on a film for real time *in situ* profiling multiple parameters.
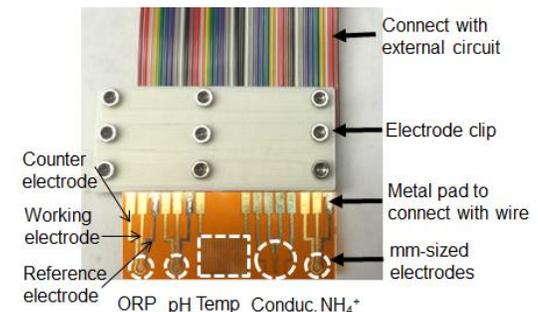


**Figure 6.** MEC setup with external circuit and data logging system.
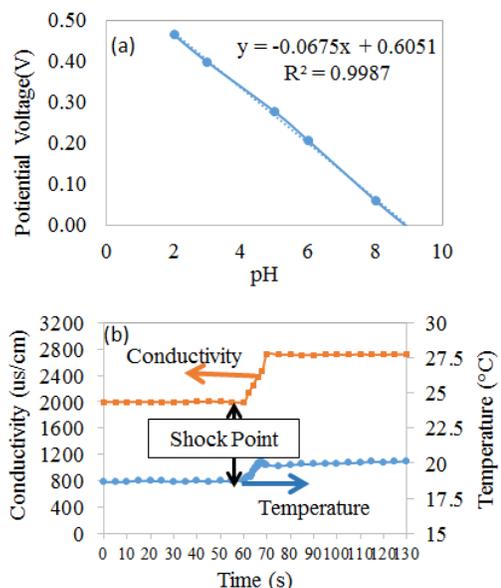


**Figure 7.** MEC calibration curve of pH (a) and MEA response to the changes of conductivity and temperature in water solution (b).

**Applying the MEC profiles for understanding migration fates of nutrients and hypoxia occurrence.** Based on the MEC profiles, the PIs have developed micro-environmental kinetic models that include mass transfer, substrate utilization by cell growth, substrate generation by cell lysis, and substrate consumption by endogenous respiration as shown below:

$$\frac{\partial S}{\partial t} = \frac{1}{L^2} \frac{(Ds \bullet L^2 \frac{\partial S}{\partial L})}{\partial L} - \frac{\mu_{max}}{Y_S} \bullet \frac{S}{K_S + S} \bullet \frac{O}{K_O + O} \bullet X + K_{LYS} \bullet X - K_{RES} \bullet \frac{S}{K_S' + S} \bullet \frac{O}{K_{OD} + O} \bullet X$$

where *dS/dt: the contaminant concentration (e.g. nitrogen, phosphorous in this project) change with time; L:the depth of the sample point; D$_s$: the diffusion constant; μ: the specific cell growth rate; O: oxygen concentration, X: the biomass concentration; K$_{LYS}$: the cell lysis constant; K$_{RES}$: the cell endogenous respiration constant; and K$_{OD}$: cell delay half-saturation constant.* The MEC monitoring encompasses the horizontal/vertical profiles of various physical-geo-chemical parameters that are responsible for the migration of contaminants in water and sediment, which will substantially enhance the information required for model simulation.

In this project, the MEC profiles in the water and sediment phases was integrated with the models developed to predict the effects of environmental conditions on the migration fates of inorganic/organic contaminants, nutrients, and oxygen at the downstream of waste discharge points (e.g. high/low COD and nutrient concentrations). Unlike the traditional oxygen models that suffer the lack of spatial and temporal readings, the multiple MEs on the horizontal and vertical directions on MECs provide the sufficient resolution to enhance model accuracy. The extrapolated location and occurrence of eutrophication and hypoxia will provide the timely restoration solutions for site cleanup before the irreversible deterioration.

Based on the lab-scale MEC tests, profiling nutrient content and hypoxia occurrence in field tests will be planned in the future study such as along the depth in the Swan Lake and the Fenton River.

**Project technology transformation.**

Public dissemination of real-time monitoring in natural water resources using MEC technology is absolutely critical given the slow acceptance of new technologies that deliver commodities. Both the PI and Co-PI are part of a growing "Water Consortium" at UConn, which aims at supporting works on water and environmental sustainability. The team has disseminated this project through active publication in journals (two journal papers under preparation) and presentations at professional conferences (e.g. two New England Environmental Conferences) based on the MEC lab tests and model simulation.

The team has been dedicated to transforming the real-time *in situ* monitoring technology by working closely with the United Water Inc., a global leading environmental engineering company. Over fifty water quality related institutes/municipalities/government agencies/private owners have been interviewed regarding MEC technology and real-time profiling technology. The feedbacks are overwhelmingly positive. We have identified the urgent needs for MECs technology in water treatment/wastewater monitoring. The collaboration with industrial partners for field tests and pilot-scale tests will further validate MEC technology.

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