Infrared sensors for environmental and biomedical applications

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ABSTRACT

Monitoring water quality by detecting chemical and biological contaminants is critical to ensuring the provision and discharge of clean water, hence protecting human health and the ecosystem. Among the available analytical techniques, infrared (IR) spectroscopy provides sensitive and selective detection of multiple water contaminants. In this work, we present an application of IR spectroscopy for qualitative and quantitative assessment of chemical and biological water contaminants. We focus on in-line detection of nitrogen pollutants in the form of nitrate and ammonium for wastewater treatment process control and automation. We discuss the effects of water quality parameters such as salinity, pH, and temperature on the IR spectra of nitrogen pollutants. We then focus on application of the sensor for detection of contaminants of emerging concern, such as arsenic and Per- and polyfluoroalkyl substances (PFAS) in drinking water. We demonstrate the use of multivariate statistical analysis for automated data processing in complex fluids. Finally, we discuss application of IR spectroscopy for detecting biological water contaminants. We use the metabolomic signature of *E. coli* bacteria to determine its presence in water as well as distinguish between different strains of bacteria. Overall, this work shows that IR spectroscopy is a promising technique for monitoring both chemical and biological contaminants in water and has the potential for real-time, inline water quality monitoring.

Keywords: infrared spectroscopy, wastewater, quantum cascade laser, sensing, principal component analysis, PFAS

1. INTRODUCTION

1.1. Importance of monitoring water quality

Water is essential for supporting life on Earth. While about 70% of Earth's surface is covered with water, only 2.5% of the water is known to be fresh.[1] With the limited amount of fresh water, it is crucial to keep our fresh water clean and maintain its quality. However, the increase in water pollution due to the world's growing population and industrial activities poses a substantial threat to the world's water quality. To efficiently clean up water pollutants with appropriate water treatments, it is critical to identify and monitor the contaminants in water.

1.2. Chemical and biological contaminants in water

To ensure drinking water safety, the US Environmental Protection Agency (EPA) established minimum water quality standards that all public water systems must adopt. EPA requires screening of chemical and biological contaminants in water, including nitrogen pollutants, per- and polyfluoroalkyl substances (PFAS), and arsenic as chemical contaminants, and *E. coli* bacteria as a biological contaminant.

A. Nitrogen pollutants. Nitrogen pollutants dissolved in water are commonly found as nitrate, ammonium, nitrite, and organic nitrogen. These nitrogen compounds are generally introduced into water through runoff of fertilizers, animal manures, and human and industrial wastes.[2] Since high levels of toxic nitrogen pollutants in water have detrimental effects on humans, aquatic life, and the environment (i.e., eutrophication and algae blooms), the contaminants in water must be measured and treated properly.

Removal of nitrogen contaminants from water is a particular pain point for municipal wastewater treatment plants (WWTPs). Removal of nitrogen is a multi-step biological process that requires aeration. Aeration is commonly achieved by means of industrial blowers moving large volumes of air through bubblers within an aeration basin. The total electrical energy used by wastewater treatment systems in the U.S. is 82.8 million kWh per day, or 30.2 TWh/yr.[3] Powering the aeration equipment requires anywhere between 50% and 75% of the total WWTP energy consumption.[4] Our primary focus is on the development of nitrogen sensors for process control and optimization within the wastewater treatment industry. Integration of a real-time, in-line nitrogen sensor for process control offers multiple economic and societal benefits.

Enabling process control at the wastewater treatment plants will result in substantial energy savings. The municipal water treatment sector consumes 2% of total US electric power.[3] Aeration accounts for more than 50% of wastewater treatment electric power consumption; potential US electric power savings from implementation of this technology for improved process control amount to $600M/year. This would result in a reduction of greenhouse gas emissions of about 4M metric tons of CO_2 per year from reduced electric power usage. Nitrogen sensor development will be discussed in section 2.1

B. Contaminants of emerging concern (CEC). Over the past decades, PFAS have received particular attention as contaminants of emerging concern (CEC) due to their persistent and bio-accumulative nature and high toxicity to human health and the ecosystem[5]. Exposure to PFAS can lead to adverse human health outcomes, such as low infant birth weight, thyroid hormone disruption, and cancer[6]. To mitigate human exposure, the EPA released a Drinking Water Health Advisory for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the two most commonly detected PFAS.[7]

Another example of CEC is arsenic, a naturally occurring element in groundwater that poses threats to human health. Arsenic contributes to serious water contamination issues in South-East Asia[8], it has also been considered to be among the highest risk drinking water contaminants in the US[9]. Arsenic in nature can be present in two oxidation states, As(III) and As(V).[10] Prolonged exposure to arsenic can cause leuko-melanosis, neuropathy, black foot disease, and cancer[11,12,13].

Discussion of our developments towards arsenic and PFOA monitoring will be presented in section 2.2.

C. Biological contaminants. To meet EPA standards, public water supply (PWS) operators are required to screen water for coliform bacteria. Although coliform bacteria are generally harmless, it is important to test for the presence of coliform species in water because their presence indicates that there is a possibility of fecal pollution, which might contain pathogenic species. A widely known species of coliform bacteria that indicates fecal contamination with the possible presence of pathogens is *E. coli.* US EPA requires testing of total coliform and *E. coli* in potable water because the presence of *E. coli* serves as an indication of fecal pollution. Our preliminary research focused on in-line detection of bacterial contaminants in water relies on metabolomic analysis and will be discussed in section 2.3.

1.3. Analytical methods for detection of water contaminants

Traditional methods of analysis for water contaminants involve “grab samples” submitted for laboratory analysis, often requiring expensive instruments with well-trained personnel. Grab-sampling is time-consuming and increases risks of out-of-control events. Generally, a lack of availability of reliable in-line sensors in the water treatment industry hinders the industry’s ability to implement automation and process control, leading to inefficient resource management.

Some commercially available sensors target in-line applications. Examples of real-time nitrogen sensors include wet chemical analyzers, ion-selective electrodes (ISEs), and UV sensors. Although there are several advantages to using wet chemical analyzers, their disadvantages include the use of chemical reagents, which produce toxic waste. ISEs do not require toxic reagents, but they are prone to a signal drift due interferents present in water and require frequent calibration. Similarly, UV sensors are hindered by optical and ionic interferents.[14]

Portable electro-analytical sensors such as SafeGuard™ that measure As(III) and total arsenic (by reducing As(V) to As(III)).[15] However, the need for frequent calibrations by trained personnel limits their application in the field.

As to the PFAS detection, there are currently no commercially available sensors.

Methods for total coliform detection are reviewed in the literature[16,17], among which fluorescence spectroscopy is mentioned as a promising in-line methodology for measurement of bacteria/coliforms in drinking water[18]. Yet, commercially available inline water sensors are limited to monitoring only a handful of contaminants, and water samples have to be routinely sent to laboratories for bio-chemical analyses.

Here, we report our R&D efforts that target implementation of a universal bio-chemical sensor for inline monitoring at drinking and wastewater treatment facilities.

1.4. Infrared spectroscopy for detecting chemical and biological contaminants in water

Max-IR Labs develops IR-based multi-analyte sensors employing tunable quantum cascade lasers (QCLs) for real-time, inline detection of chemical and biological water contaminants. IR spectroscopy is a highly sensitive and selective analytical technique that enables real-time, direct detection of target molecules as it measures the unique absorption frequency of molecules in the mid-IR region. While traditional lab-based systems rely on bulky Fourier-transform infrared (FTIR) spectrometers that are sensitive to vibrations, QCLs enable novel sensor designs due their small size, weight and power (SWaP) advantage, high spectral emission power, and ruggedness.

Application of IR spectroscopy to water analysis is extremely challenging because water strongly absorbs IR radiation. Figure 1A illustrates attenuation of IR radiation as it passes through water with increasing path lengths. To overcome this challenge, Max-IR Labs utilizes attenuated total reflection (ATR), a mechanism for radiation transfer through a waveguide in contact with water rather than through water. Figure 1B shows typical ATR-IR spectra of PFOA and two oxidation states of arsenic in water. In the ATR regime, as IR light travels through a waveguide, the sensing is done by means of an evanescent field at the boundary between the waveguide and the surrounding medium. This field can detect molecules within a few microns of the waveguide surface. This ATR sensing mechanism is shown in the inset of Figure 1B. With this technique, IR radiation that carries a spectroscopic signature of water contaminants can reach the detector without being absorbed in water. Moreover, since ATR-IR spectroscopy is specific to bacterial metabolomic products (i.e., carbon dioxide, lactate, ethanol, acetate), it allows identification and detection of bacteria (biological contaminant) in water.

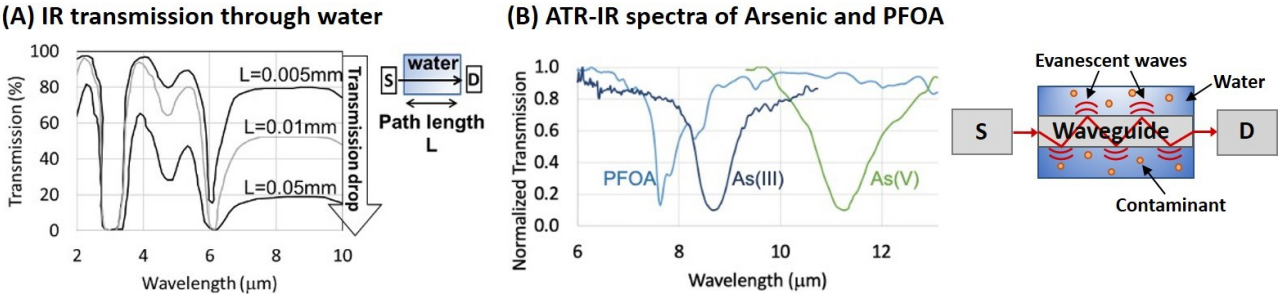


Figure 1. (A) IR transmission spectra through water with path length (L) ranging from 0.005 mm to 0.05 mm, showing a strong absorption of IR radiation in water. Inset: a schematic diagram of the experimental setup. (B) Normalized ATR-IR spectra of PFOA, arsenite (As(III)), and arsenate (As(V)) in water, demonstrating high selectivity towards those water contaminants. Inset: a schematic diagram of Max-IR Labs' ATR-IR sensor design. Note: S represents a light source and D represents a detector.

In this manuscript, we report some key results in the experimental study done at Max-IR Labs to evaluate selectivity and sensitivity of ATR-IR spectroscopy in detecting chemical and biological water contaminants. The chemical contaminants we focus on are ammonium, nitrate, PFOA, and arsenic, and the biological contaminants are different *E. coli* strains.

2. EXPERIMENTS AND RESULTS

2.1. Nitrogen monitoring: the effect of salinity, pH, and temperature

We develop our sensors tailored to the needs of water industry applications. Max-IR Labs' real-time nitrogen sensor will enable wastewater treatment automation and process control. Present-day grab-sampling practices lead to manual operation of the aeration blowers, inefficient energy management, and a high risk of out-of-control events. For wastewater treatment applications, we must ensure that the sensor is stable during *all seasons* and *over ranges of pH and salinity*. In this section, we discuss the effects of salinity, pH, and temperature on the IR spectra of nitrogen contaminants in the form of nitrate and ammonium.

2.1.1. Salinity variation

Salinity is the measure of dissolved salts in water, generally expressed in parts per thousand (ppt). Salinity is an important factor to consider in wastewater treatment[19–21], where salinity levels vary depending on the water source. For instance, salinity levels vary from 0 to 0.5 ppt for fresh water and from 1 to 30 ppt for brackish estuaries.[22]

To determine the effects of salinity on the IR spectra of nitrate and ammonium, ATR-IR spectra of nitrate (Figure 2A-B) and ammonium (Figure 3A-B) were taken at different salinity levels ranging between 0.5 and 30 ppt (using Nicolet™ iS50 FTIR spectrometer from Thermo Fisher Scientific). Using a salinity meter (Pro30 handheld salinity instrument from YSI.), the salinity of both nitrate and ammonium solutions were adjusted with a saturated NaCl solution at a constant NO_3-N (63 parts per million (ppm)) or ammonium (71 ppm) concentration. Interestingly, in addition to nitrate (Figure 2A) and ammonium (Figure 3A) IR absorption peaks, an IR peak at around 1635 cm^{-1}, representing $NaCl(H_2O)_n$ complexes, was observed (see Figures 2B and 3B). While the background for the ATR-IR spectra of nitrate and ammonium varied across the salinity levels, the IR peak areas for both nitrate and ammonium did not change significantly as illustrated in Figures 2C and 3C. Furthermore, there was a linear relationship between the peak area of $NaCl(H_2O)_n$ complexes and the salinity

in the samples (Figures 2D and 3D). The results indicate that the salinity levels from 0.5 to 30 ppt have no significant impact on the IR peak areas of nitrate and ammonium, and also show that the salt content in a sample can be quantitatively estimated using the IR peak of $NaCl(H_2O)_n$ complexes. Max-IR Labs utilizes these changes in the IR absorption bands due to the presence of salt to monitor salinity values.

Figure 2. Averaged ATR-IR spectra of NO_3-N solution (63 ppm) showing the IR absorption peaks of (A) nitrate and (B) $NaCl(H_2O)_n$ complexes at salinity levels ranging from 0.5 to 30 ppt. (C) A plot of IR peak area of nitrate as a function of salinity. (D) A plot of IR peak area of $NaCl(H_2O)_n$ complexes as a function of salinity. Each spectrum or data point in A-D represents an average of 5 spectral scans taken at each salinity level. ATR-IR spectrum of water was used as a reference.

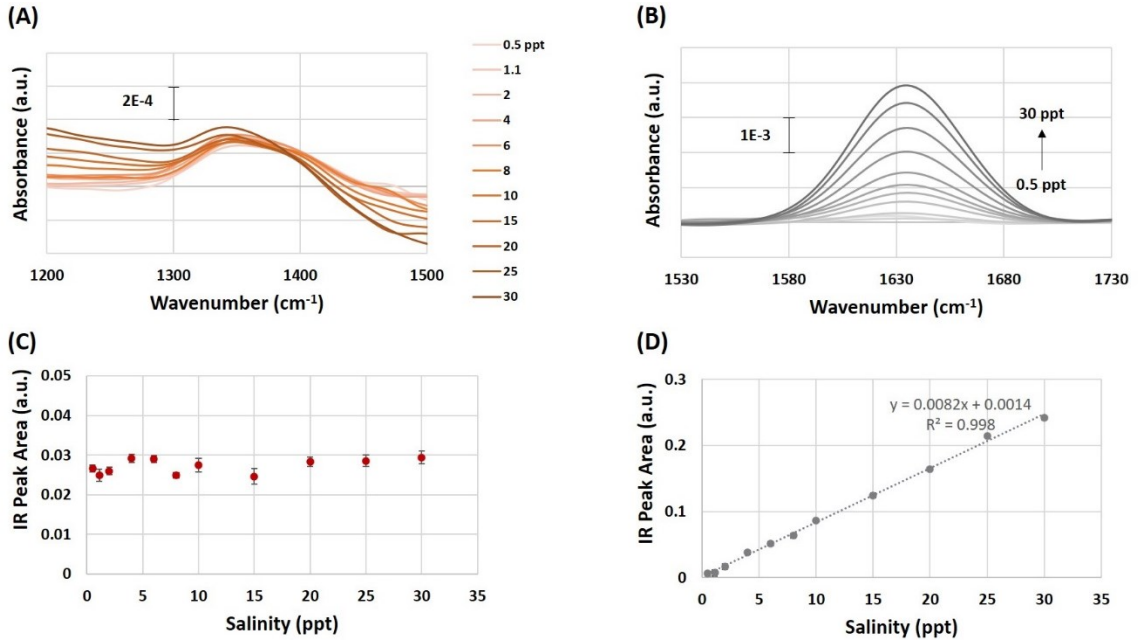
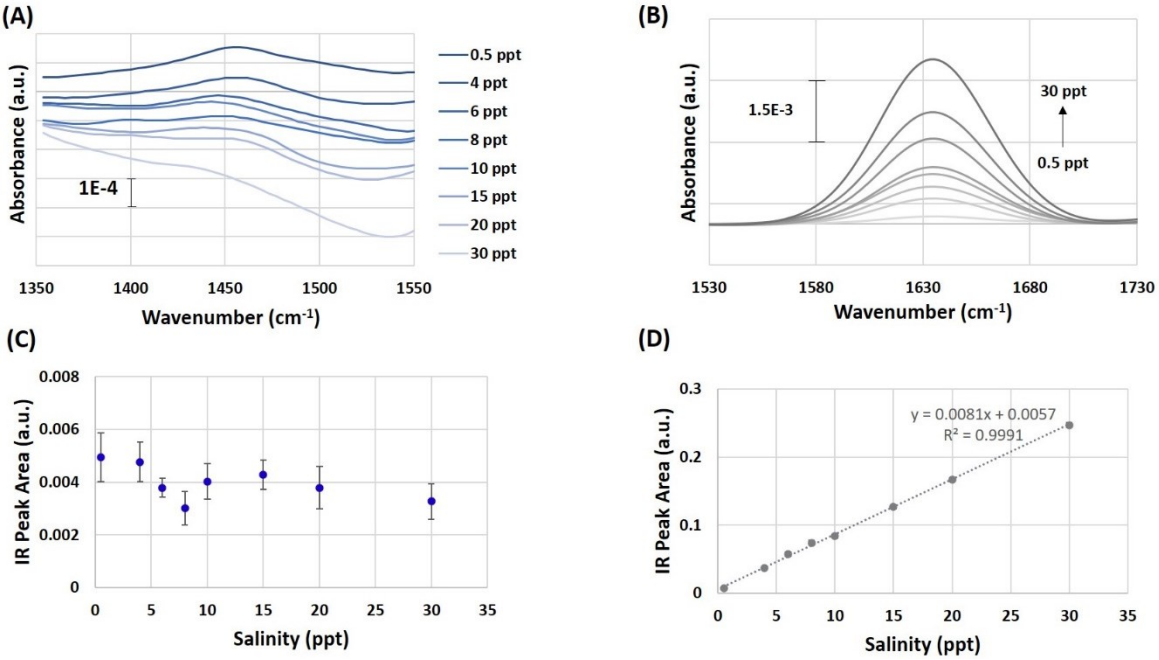


Figure 3. Averaged ATR-IR spectra of ammonium solution (71 ppm) showing the IR absorption peaks of (A) ammonium and (B) $NaCl(H_2O)_n$ complexes at salinity levels ranging from 0.5 to 30 ppt. (C) A plot of IR peak area of ammonium as a function of salinity. (D) A plot of IR peak area of $NaCl(H_2O)_n$ complexes as a function of salinity. Each spectrum or data point in A-D represents an average of 5 spectral scans taken at each salinity level. ATR-IR spectrum of water was used as a reference.



2.1.2. pH variation

The EPA suggests that the pH of freshwater should fall in a range between 6.5 and 9.[23] As such, it is important to ensure that Max-IR's sensor reliably detects nitrogen under various pH conditions. To determine the effect of pH on nitrate and ammonium, ATR-IR spectra of nitrate and ammonium were taken at different pH levels ranging 6 to 9 (at constant nitrate and ammonium concentrations). The pH of both nitrate and ammonium solutions were adjusted by adding either 1 M NaOH or 1 M H_2SO_4 using a benchtop pH meter (Thermo Scientific). The IR peak areas of both nitrate and ammonium were then plotted as a function of pH (see Figure 4). For nitrate, no significant peak area changes were observed (Figure 4A). For ammonium, however, the peak area decreased as the pH increased (Figure 4B), which was expected since more ammonium transforms into ammonia as the pH level increases. Owing to consistent behavior of the ammonium absorption peaks at varying pH values, the correction of the ammonium concentration values in our sensor is based on these calibration curves.

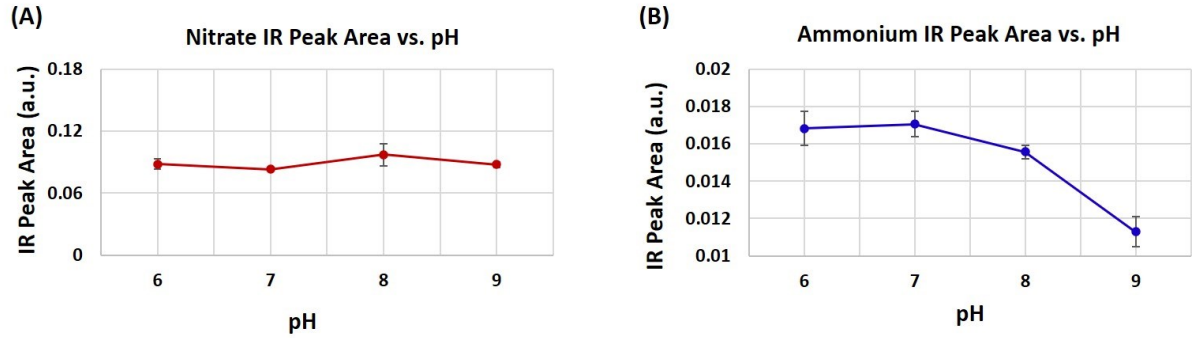


Figure 4. Averaged ATR-IR peak area of (A) nitrate and (B) ammonium versus pH level (ranging from 6 to 9). The concentration of NO_3-N and ammonium are both 200 ppm. ATR-IR spectrum of water was used as a reference. Each data point in graph A represents an average of 20 spectral scans across two samples at each pH level and each data point in graph B represents an average of 5 spectral scans at each pH level.

2.1.3. Temperature variation

Aiming at all-season operation of the sensor, we validated the temperature dependence of the IR absorption peak areas of nitrate and ammonium. ATR-IR spectra of nitrate and ammonium were taken at different temperatures ranging between 2.5 and 50°C. The temperature of nitrate and ammonium solutions were adjusted using an ice bath and a hot plate. As shown in Figure 5, the nitrate and ammonium peak areas did not change significantly with temperature variation.

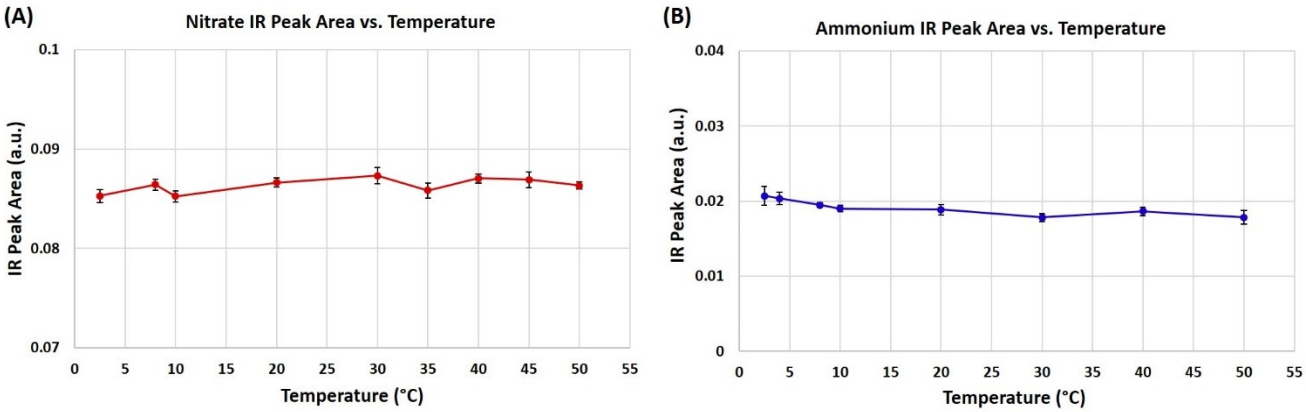


Figure 5. Averaged ATR-IR peak area of (A) nitrate and (B) ammonium as a function of temperature from 2.5 to 50°C. The concentration of NO_3-N and ammonium are 200 ppm and 250 ppm, respectively. Each data point represents an average of 10 spectral scans. ATR-IR spectrum of water was used as a reference.

2.2. Implementation of PCA for automated data processing and quantitative detection of multiple water contaminants

Beyond detection of nitrogen-based contaminants, infrared spectroscopy is a powerful tool for detection of other critical water pollutants such as PFOA and arsenite. For example, the unique IR absorption bands of PFOA, arsenite, and nitrate are shown in Figures 6A-C.

In this section we address our approach towards quantification of the contaminants when they are present in complex mixtures at low concentrations. A spectrum obtained from such mixture containing all three contaminants is shown in Figure 6D. To quantify each contaminant in the mixture in an automated manner, we utilized a software (written in Python) integrated with multivariate statistical analysis algorithm based on principal component analysis (PCA), which is a statistical data analysis technique that allows one to extract meaningful information by reducing the dimensionality of a data set and maximizing variances in the data with minimal information loss from the reduction of dimensionality.[24]

By performing PCA, we first obtained a linear calibration curve for each analyte with a known concentration using the extracted first principal component (PC1), which represents the maximum variance in the spectral dataset (Figures 6E-G). The negative PCs result from a spectral pre-processing step of normalization and background subtraction with an arbitrary origin of y-axis. The result clearly shows linear calibration curves for PFOA, arsenite, and nitrate, which are important for quantitative analyses. We then plotted the extracted PC1 for each contaminant in the mixture in each calibration plot (marked as a red dot in Figures 6E-G). This preliminary result demonstrates the capability of Max-IR Labs' automated data analysis for obtaining calibration plots and quantifying each contaminant in a complex mixture. We are currently working on optimizing and increasing the accuracy of this sensing approach by pre-concentrating the analytes in proximity to the waveguide and enlarging our spectral databases.

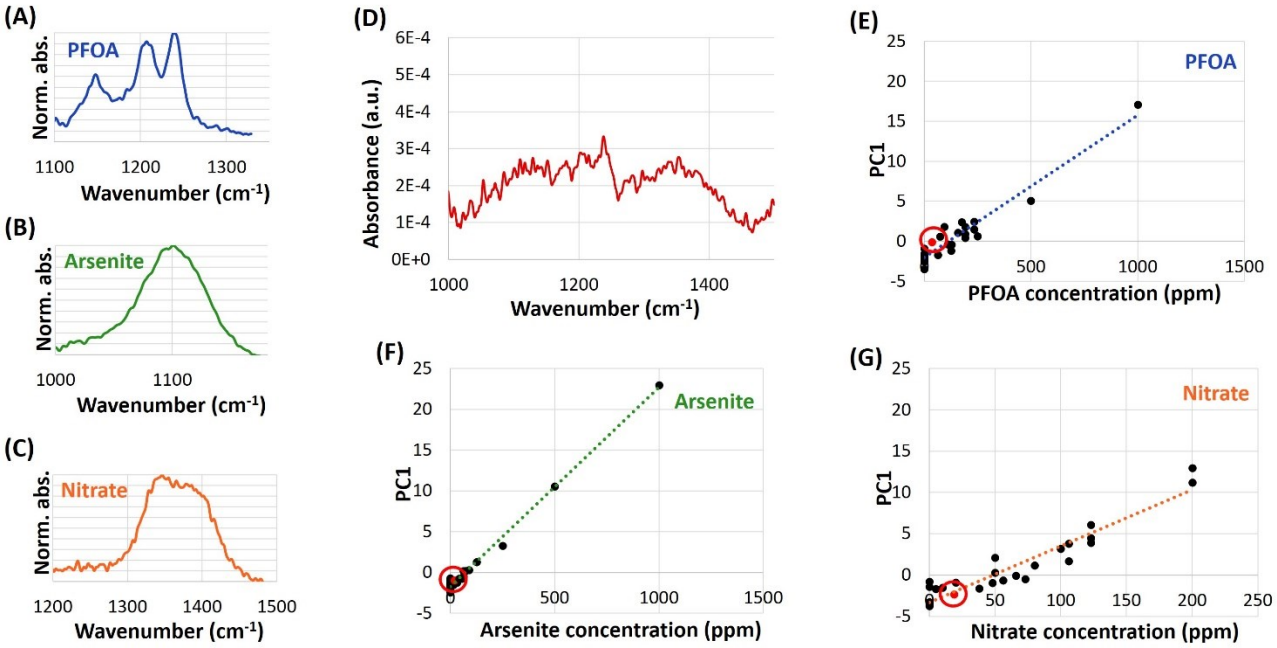


Figure 6. Normalized ATR-IR spectra of (A) PFOA, (B) arsenite, (C) nitrate. (D) An ATR-IR spectrum of a mixture with PFOA, arsenite, and nitrate (concentrations of PFOA, arsenite, and nitrate in the mixture are 37, 21, and 19 ppm, respectively). PCA analysis of ATR-IR spectra of (E) PFOA, (F) arsenite, and (G) nitrate. Calibration curve for each contaminant was obtained by plotting the first principal component (PC1) for each contaminant as a function of its concentration in water. The PC1 obtained for each contaminant in the mixture is then plotted, shown as a red dot on each plot (encircled in red).

2.3. Bacterial metabolomic analysis

IR spectroscopy is also well suited for sensing bacteria by detecting their fermentation signatures resulting from bacterial metabolism, which includes depletion of energy sources (i.e., carbohydrates and phosphates) and production of of various compounds such as carbon dioxide, lactate, ethanol, and acetate.[25] As concentrations of the end-products may vary depending on the types of bacteria and their growth conditions, spectral analysis of the bacterial growth media enables identification and detection of bacteria in water. As shown in Figure 7A, infrared analysis of a liquid growth medium of *E. coli* allows one to observe energy source depletion (negative absorption bands) and major product generation (positive absorption bands). In addition, identifying the production of acetate and lactate can help distinguish between different bacterial types. For instance, the ATR-IR spectra of metabolites produced by E. *coli* W3110 wild-type strain and W3110 *pta* mutant (lacking an enzyme required for acetate formation) in Figure 7B show that the resulting metabolomic contents clearly differ, allowing one to distinguish between the two strains of *E. coli* bacteria.

The ability to perform rapid, in-depth metabolomic analysis is useful not only in water industry, but also in medical diagnostics. Presently, Max-IR Labs looks into development of sensors for analysis of biofluids, such as urine, where specific metabolomic signature can help in providing fast and reliable diagnosis of, e.g., urinary tract infection (UTI).

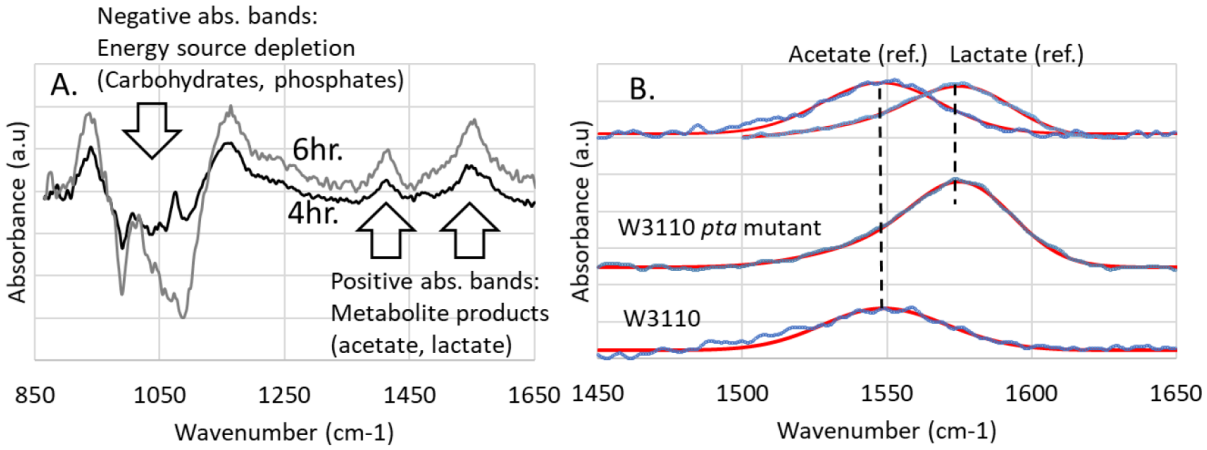


Figure 7. (A) ATR-IR spectra obtained from a growth medium of *E. coli* after 4 and 6 hours of growth. All data was referenced to spectra obtained from initial growth medium (measured prior to growth). Negative absorption bands are due to the depleted compounds (carbohydrates and phosphates) and positive absorption bands are due to the generated compounds (acetate and lactate). (B) ATR-IR spectra obtained from *E. coli* W3110 and W3110 *pta* mutant in comparison with the reference spectra of 15 mM acetate and 10 mM lactate (blue: measured spectra, red: spectral fit). Acetate and lactate metabolites are clearly identified for *E. coli* W3110 and W3110 *pta* mutant, respectively. All the spectra were produced in collaboration between Max-IR Labs and the University of Texas at Dallas.

3. CONCLUSION

In summary, we reported Max-IR Labs' key results from a study identifying and detecting water contaminants, both chemical and biological, using ATR-IR spectroscopy. To transfer this powerful technology from lab to field, we are currently developing a novel ATR-IR sensor platform integrated with QCLs for real-time, inline screening of multiple contaminants present in water.

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