# Introduction

## Detection of bacteria in drinking water

In spite of the efforts put into maintaining drinking water safe, we still face many bacterial water contaminations, from bacteria such as *Legionella, Salmonella, Escherechia* that cause gastroenteritis, skin disease and other ailments (Leclerc 2002). In the US alone, ~40,000 people are hospitalized due to microbial contamination of water, resulting in 970,000 dollars of damages annually (collier 2012). The source of these contaminations may be direct contamination of water sources, pipelines or home and institutional taps or failures in the sanitation system in bottling and water processing plants.

Water treatment plants use different methods to prevent microbial contamination of drinking water. The water may be filtered by several types of filters, chemically sanitized (by chlorination or ozonation) and sometimes even reverse osmosis is used to insure water safety. Further down the supply chain the water is sometimes re-filtered to get rid of any contaminants that arrive from piping contaminators and in some homes and institutions an in-house treatment is done using micro-filters, active carbon and UV light sanitation. In recent years we have seen more of these in-house sanitation approaches in home “water bars”. In spite of all these efforts, pathogenic bacteria sometimes reach consumers. In these cases, the local authorities would often redirect water distribution until the contamination is either disinfected or naturally subside (Ashbolt 2015, MinOH 2013, MinOH 2016, MinOH 2017).

Due to the dangers of water contamination, constant monitoring of the microbial quality of water is required. Timely identification and treatment of contaminations can prevent pathogens from reaching consumers, saving a lot of money and improving public health. The water industry uses many different methods to detect contaminations, such as turbidity measurement and culture-based selective assays that detect coliforms, fecal coliforms and fecal streptococci. These tests are done throughout the water treatment process and in many control points in pipelines. This main issue with these tests is that the time to get results is long, which means that in practice – contaminated water reaches consumers. Furthermore, the standard tests are usually not specific, or are specific only to indicators (such as coliforms) ans cannot distinctly detect pathogens such as *Legionella* and *Campylobacter.* Another standard test is the heterotrophic plate count test, which quantifies the general microbial load in the water without discriminating harmful or harmless bacteria (Rompre 2002, Edberg 2000).

Common microbiological lab methods for the detection of bacteria generally include culturing samples in selective media, in optimal conditions, followed by colony counts or turbidity measurements (Edberg 2000). Colony counts are an indication of the number of bacteria that is measured by the normalized amount of bacterial colonies on an agar plate. The number is usually normalized to colony-forming-units per ml (CFU/ml). These methods are problematic because the formation of colonies requires a long time, between 12 hours and a week. Turbidity measurements can be done without culturing but they allow only rough estimates, efficient only in exceptional cases where the microbial load is as high as 106 CFU/ml or more. These disadvantages are one of the greatest challenges in the food and water industries, and cause a threat to public health (Leclerc 2002, Hennekinne 2012). Moreover, current methods require a large team of trained personnel and lots of resources (growth media, petri dishes etc.) which makes testing expensive (Rompré 2002). Not only that, but testing is only done on small sample sizes of between 0.1-1 liter out of hundreds of thousands that pass through the system. The tests are done periodically and the majority of water is not tested. Since water contamination is unpredictable and sporadic (Cabral 2010, Frolich 2017), a failure in testing in the water industry can be identified which may undermine public health. This failure is well known but presently no high quality and affordable alternative is in use (Rompre 2002, Willemse-Erix 2009)

Several modern methods have been suggested to replace the standard. Molecular methods, based on specific DNA amplification have been shown to be highly specific to pathogens (romper 2002) but they still require a long culturing stage. More recent methods, based on nucleic acid microarray or Enzyme-Linked Immunosorbent Assay (ELISA) technology can provide highly specific results faster (within hours). However, these methods depend upon expensive reagents, instruimentation and highly trained personnel and are thus not applicable to most industrial uses. Spectral methods, based on mass spectrometry have also been proposed, especially the Matrix-assisted laser desorption/ionization (MALDI-TOF) approach. These utilize high energy laser to ionize samples, causing molecular structures to breakdown and measure the products using mass spectrometry. While this approach requires fewer expensive reagents, it remains an expensive alternaive due to the cost of the instruments. Further, the method requires culturing (albeit shorter than standard) which takes time and is limited to specific types of bacteria. There still remains a need for a rapid, accurate and quantitative method for the detection of bacteria in drinking water that is cheap and reliable. In this work we propose the application of Raman and fluorescence spectroscopy for the identification and quantification of bacteria in drinking water and the food industry.

## Raman spectroscopy

### Scientific background

Raman spectroscopy is a modern analytical tool, which can deliver rapid results at a relatively low cost. The method is based on Raman Shift, a phenomena first described by CV Raman in 1928. When photons of a specific wavelength interact with a molecule some of the photons go through a Raman Shift, where the photons’ energy (and wavelength accordingly) are altered. This is because some of the photons wave energy is transformed into vibrational energy in the molecular structure (Bernhard S:1995). The shift is different between molecular structures and bonds, so the effect is specific and a molecular “fingerprint” can be assembled (Bernhard, Kaiser). Raman shift is dependent upon the Polarizability of a substance, i e the flexibility of its electron cloud. This is because the energy is transferred via the electron cloud, and in a low polarizability substance the photons cannot transfer the energy. Water molecules (H2O) have very low polarizibality, and thus a minute Raman signal, making them an excellent background for Raman spectroscopy (Stockel 2015).

Raman shift is measured using a light spectrometer which measures the light’s wavelength after it has gone through a sample and converts it into a digital signal for computer processing (Stockel 2015). The digital spectrum displays the intensity of light at each wavelength that is longer than the original transmitted light (which is cutoff from the signal). This light had undergone Raman Shift (fig. XXX). The wavelength can be displayed as absolute wavelength in nanometers, but is usually converted into energy shift in units of cm-1.

Figure 1 Spectral fingerprint of ethanol