Training Program on Sustainable Natural and Advance Technologies and Business Partnerships for Water & Wastewater Treatment, Monitoring and Safe Water Reuse in India

AIMEN Sensors: VFA and Pathogens

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aimen TECHNOLOGY CENTRE

PAVITR

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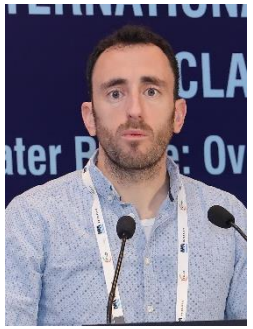
Introduction to the authors



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Learning objectives

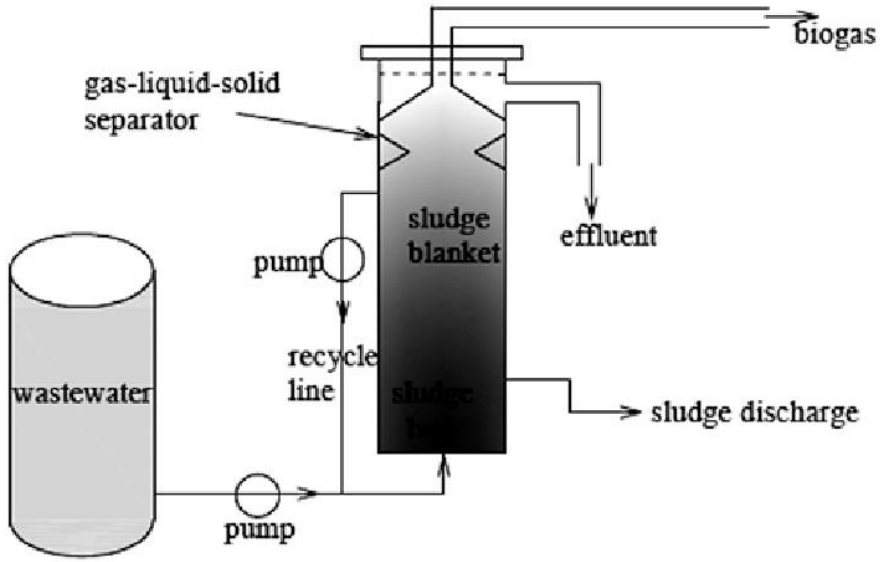
At the end of this session, participants will:

- Understand the application of the technology
- Acknowledge the need for this technology
- Understand the sensors working principle
- Learn how to use the devices and perform a measurement
- Review the validation and implementation activities in Aligarh (AMU site in PAVITR project)

Agenda of the session

Time	Content
5 min	Introduction to the session
10 min	Introduction to the technology (background overview, principles, performance expected, appropriateness)
30 min	Design of the technology (key considerations, basic calculations, key formulas, etc.)
20 min	Break.
10 min	Assembly and implementation
10 min	Operation and maintenance
10 min	Example: the PAVITR pilot
10 min	Homework: exercise to design/implement the technology for a case study
5 min	Final remarks

Introduction to the technology



Nnaji, Chidozie. A Review of the Upflow Anaerobic Sludge Blanket Reactor 10.1080/19443994.2013.800809

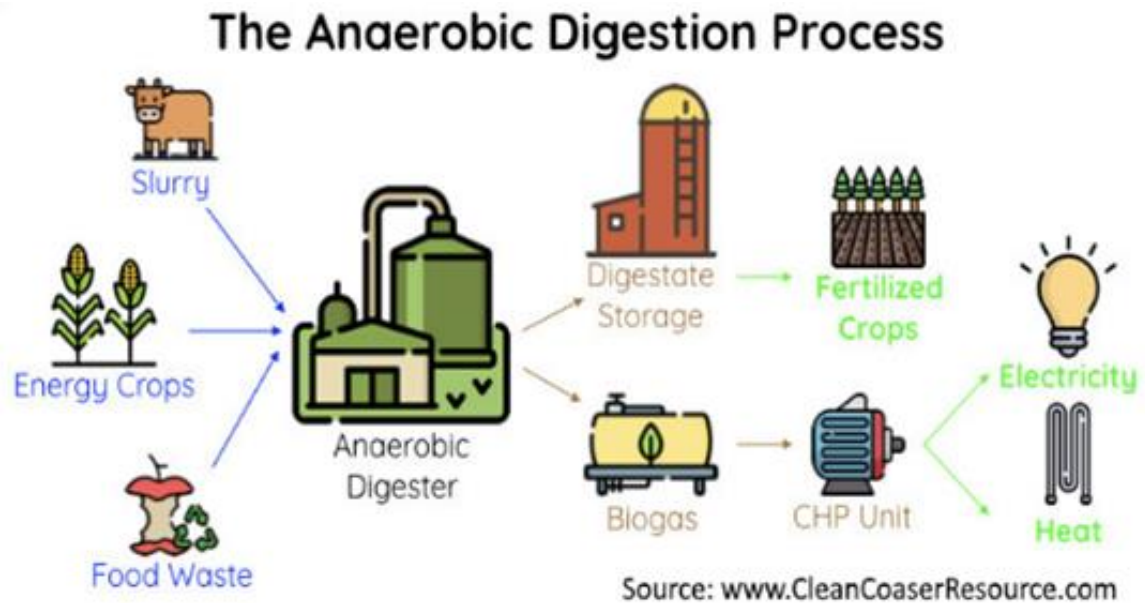
VFA SENSOR

VFA

Anaerobic digestion (AD) is a natural process in which **microorganisms** break down organic matter in absence of oxygen (EPA, EEA).

- **Wastes in AD**: animal manure, food wastes, fats & oils, sewage sludge, other industrial organic wastes.
- **By-products of AD**: valuable products as biogas, nutrients, and raw material for other processes → Circular (bio)economy
- **Biological process** control of conditions in the reactor:
  - Physic-chemical parameters
  - Feedstock properties
  - Compounds into digester:

    → **Volatile Fatty Acids**



Source: www.CleanCoaserResource.com

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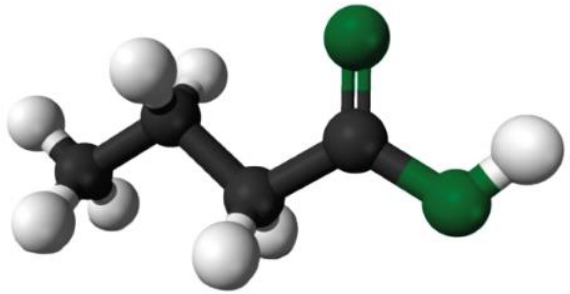
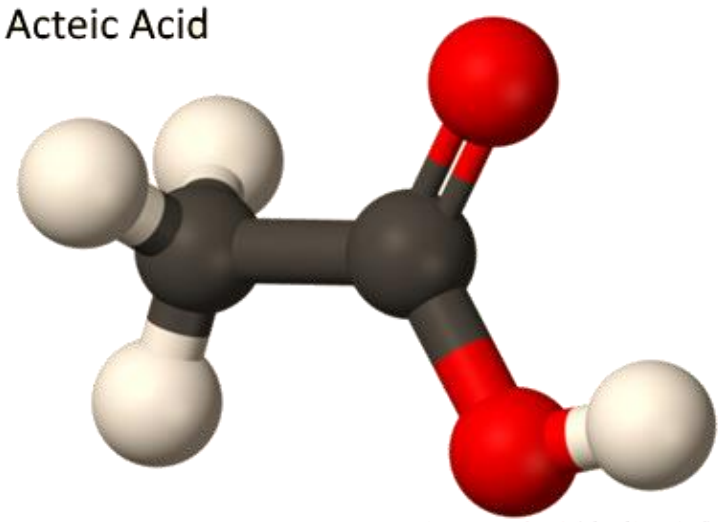
VFA

Volatile Fatty Acids (VFA) are organic compounds composed of short-chain fatty acids (C2 – C6 carboxylic acid), such us, acetic , propionic or butyric acid, among others.

- **Presence in AD**: Produced in initial septs of AD (acidogenic phase), VFA are the pre-intermediate for the methane production.
- **Control VFA in AD:** Monitoring VFA concentration in the reactor → indicative of AD process .
- **Interest in VFA production**: Carbon source for products: Biopolymers (PHAs), Medium chain fatty acids, biofuels, hydrogen generation…





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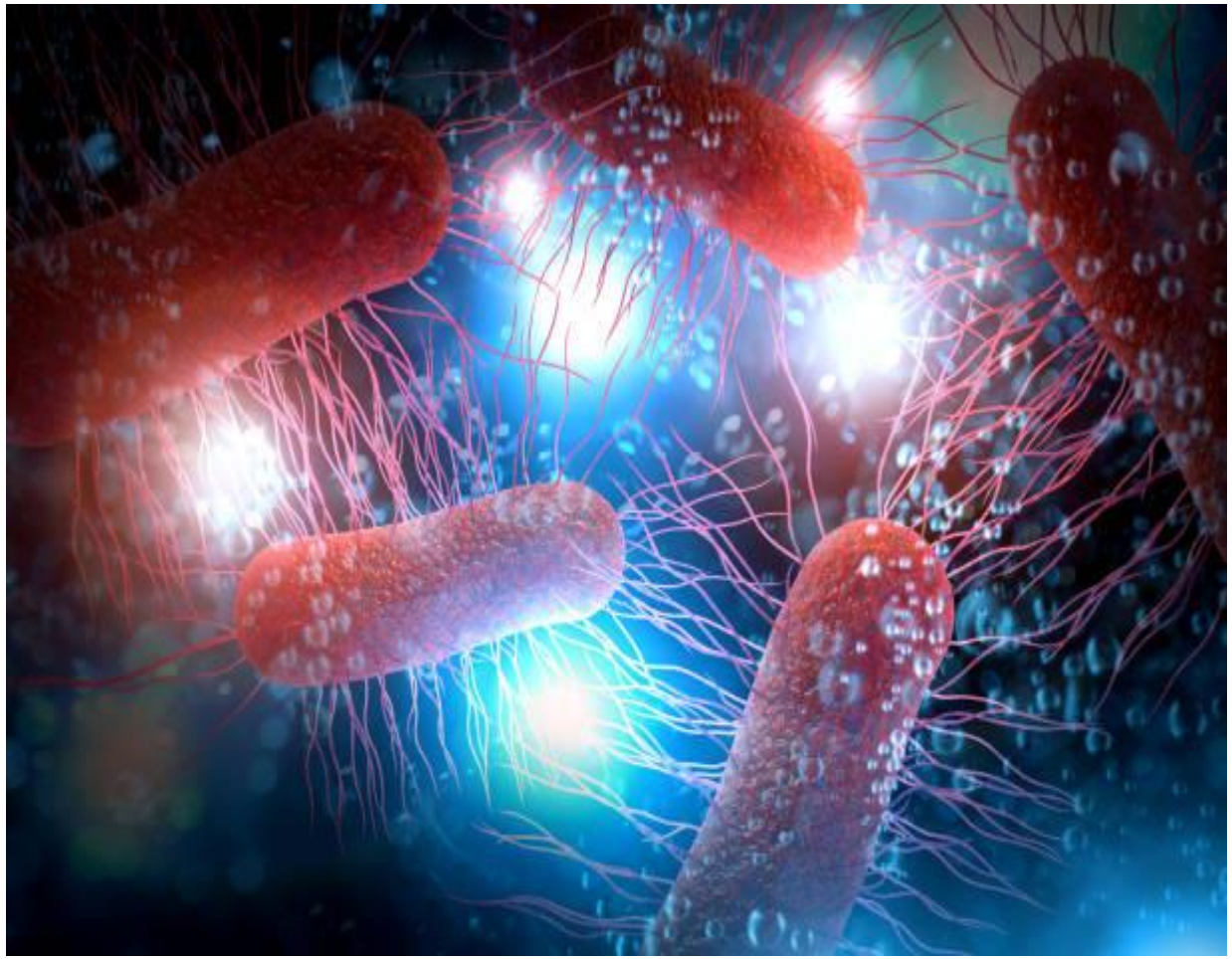

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PATHOGEN SENSOR

Pathogen Sensor

The necessity of providing a quality treated water -> absence of pathogens

- Water Quality control required for avoiding healthy issues in the population
- **Coliforms** and ***Escherichia coli*** – mainly monitored pathogens → indicator of faecal pollution in the water.
- Legislation: (Directive (EU) 2020/2184, Regulation (EU) 2020/741, Indian Standard (IS) 10500 : 2012:, Directive 2006/7/EC, WHO).

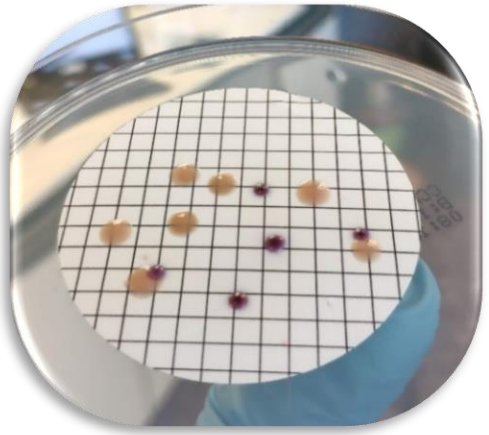
Use of water	*E. Coli* limit (cfu/100 ml)	comments
Human consumption	0	*Shall not be detectable in any 100 ml sample*
Agriculture irrigation	10 - 10000	*According to crop category*
Bathing water	250 - 1000	*According to water category*

Pathogen Sensor

The necessity of providing a quality treated water -> absence of pathogens

- *E.coli* determination:
  - Standard methods requires specific materials and equipment.
  - Qualified staff
  - Analysis time: 21± 3 h at 36 ºC for the correct determination.
  - Escherichia coli is regarded as a key parameter as an indicator of fecal pollution in water. for controlling the water quality for drinking and irrigation purposes

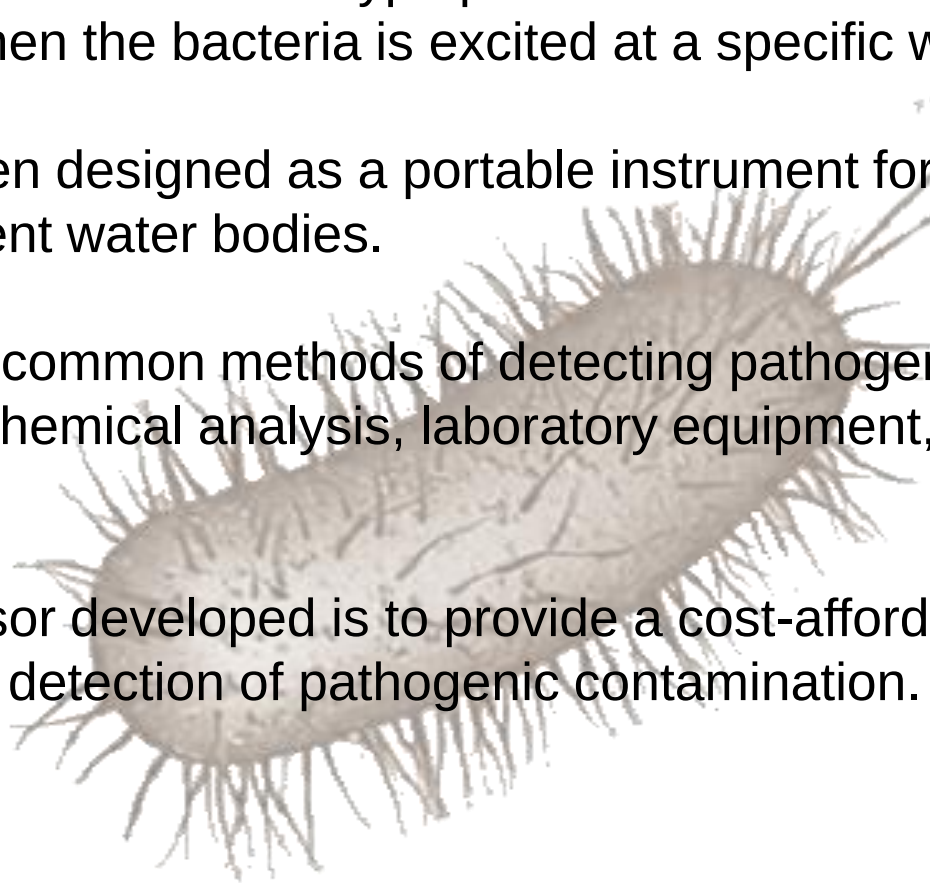
Image by DCStudio on Freepik



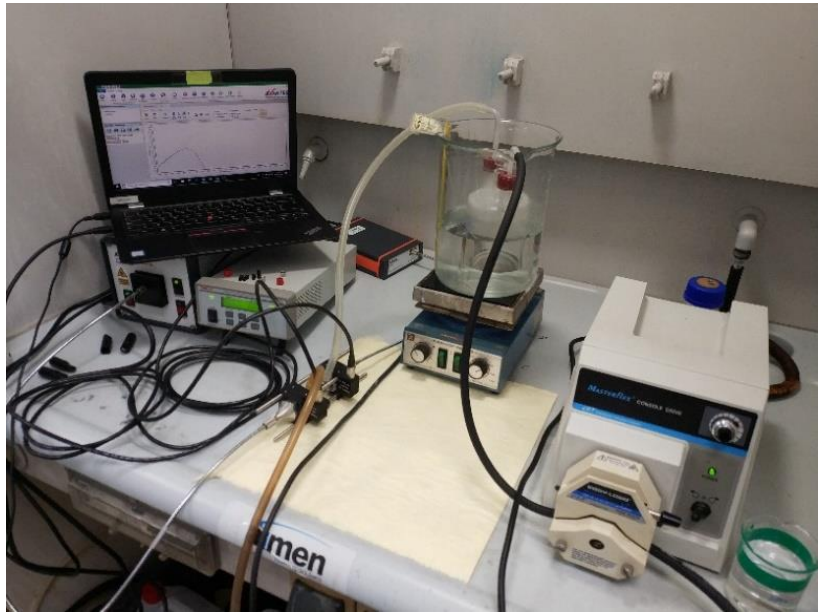
Pathogen Sensor

PAVITR

- Its working principle is based on Tryptophan-like fluorescence emitted by E. coli and other pathogens when the bacteria is excited at a specific wavelength.
- The sensor has been designed as a portable instrument for the field determination of pathogens in different water bodies.
- Currently, the most common methods of detecting pathogenic contamination in water requires thorough chemical analysis, laboratory equipment, qualified staff and long incubation periods.
- The aim of the sensor developed is to provide a cost-affordable and time-saving alternative for early detection of pathogenic contamination.



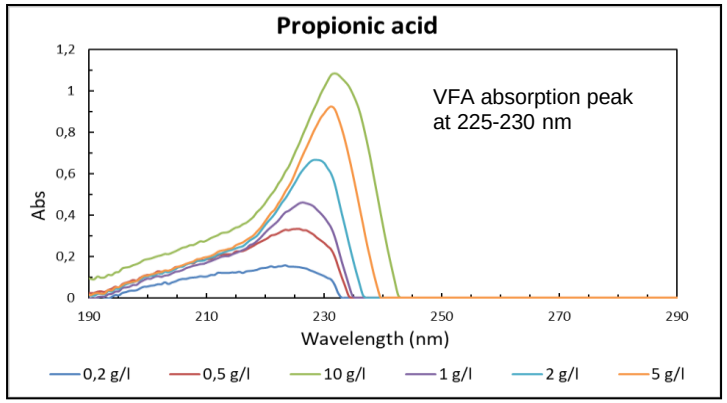
Design of **the technology**



VFA SENSOR

VFA sensor

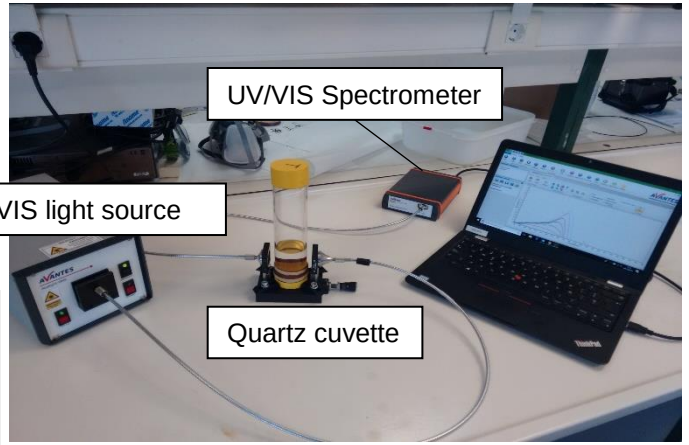
Optical sensor based on UV-VIS spectroscopy in liquid sludge samples (decanted, filtered, diluted, etc.). Technology already validated in AIMEN laboratories.



UV/VIS photodiodes/spectrometers

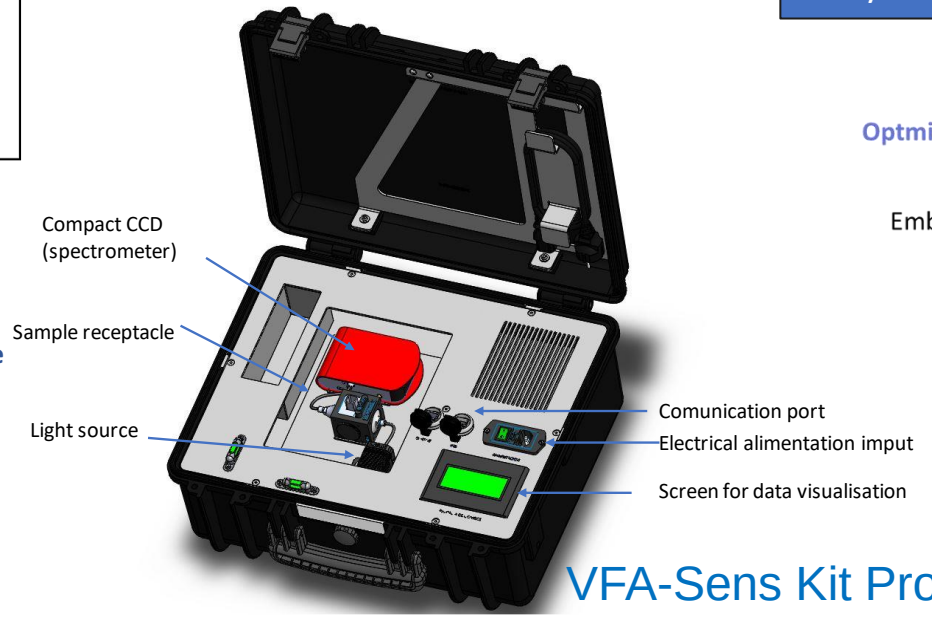
UV/VIS compact light sources

PAVITR: optmized cost effective system



VFA spectroscopy sensor laboratory setup

In the frame of PAVITR project UV-VIS spectroscopy optical sensor will be integrated in a suitcase. A rough portable kit for in situ measurement, able to collect and transfer data analysed.



VFA-Sens Kit Prototype

Optmized software and hardware

Embedded system (PC/104…)

VFA sensor

Sensor development for the live measurement of VFA concentration in the UASB to control the process stability and biogas production.

- Optical sensor based on UV-VIS spectroscopy
- Samples: liquid sludge phase and gas sample tests

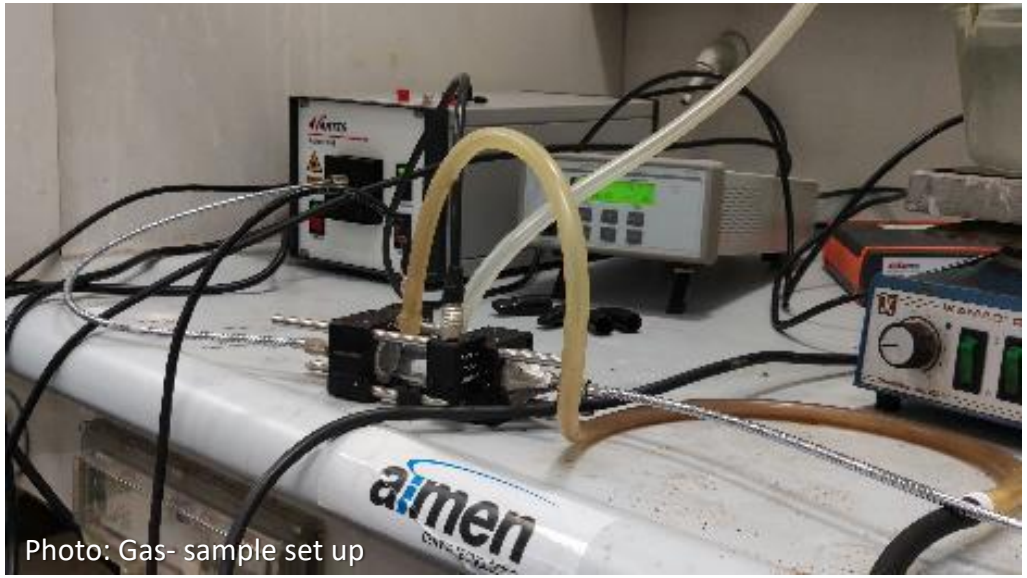


Photo: Gas- sample set up

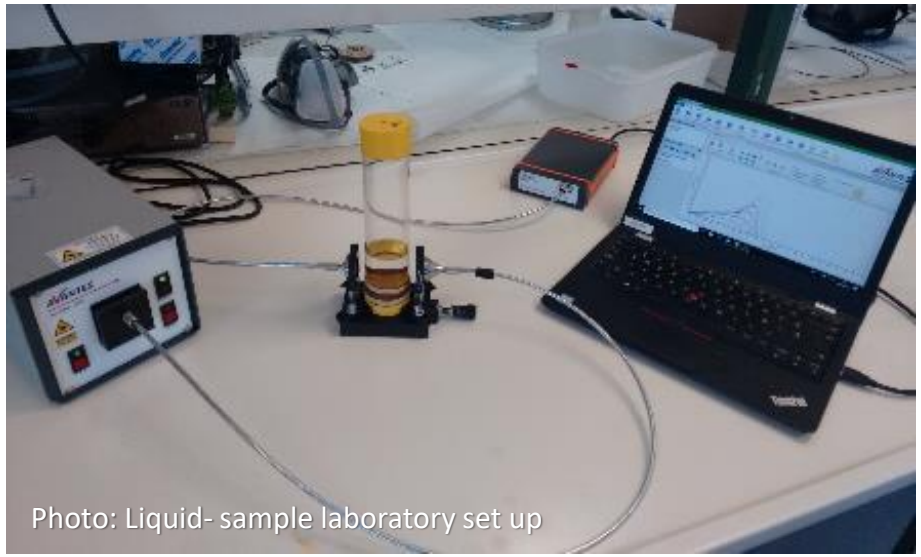


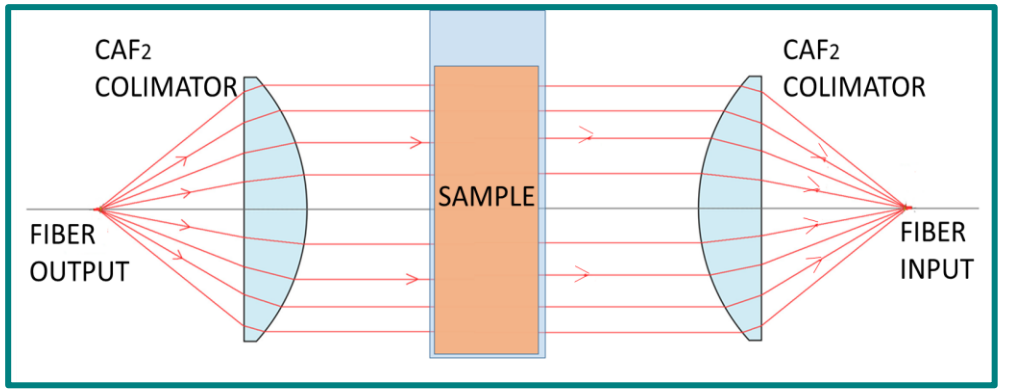
Photo: Liquid- sample laboratory set up

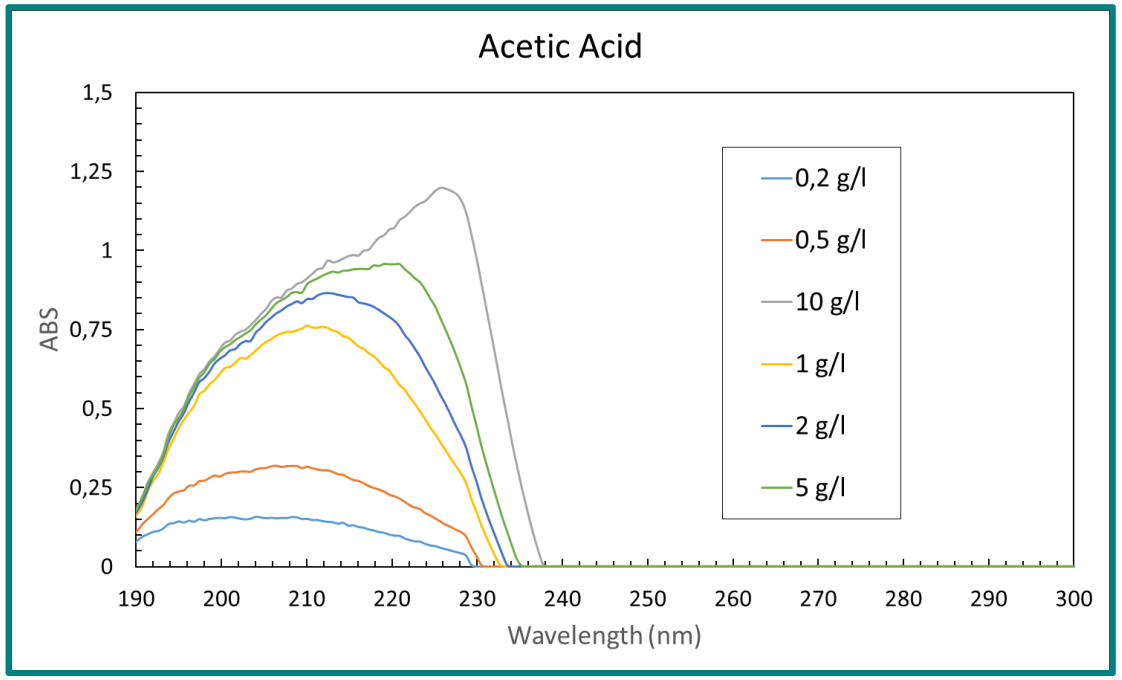
- Components of the **VFA sensor**
  - UV/VIS light from a Deuterium/Halogen lamp
  - Fiber to guide light (1000 um)
  - Glass cuvette: liquid or gas samples
  - Avantes UV/VIS spectrometer

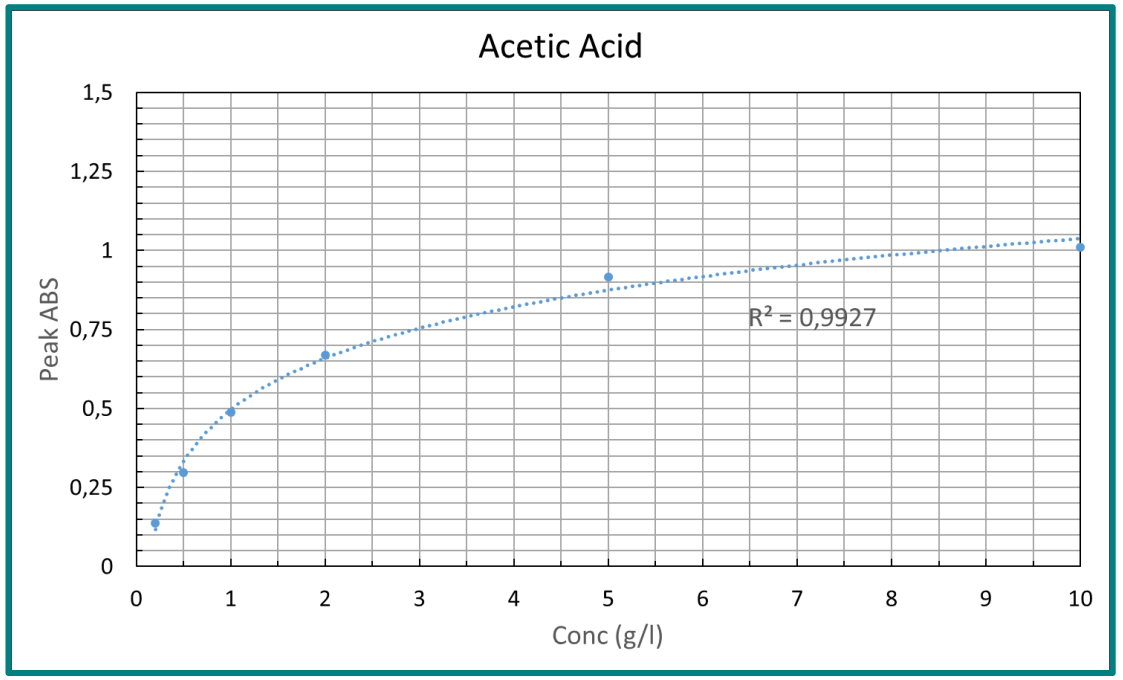
VFA sensor

VFA SENSOR

The initial goal was to transfer preliminary spectroscopy results from liquid phase to gas phase, in order to avoid sludge turbidity and complex filtering processes.

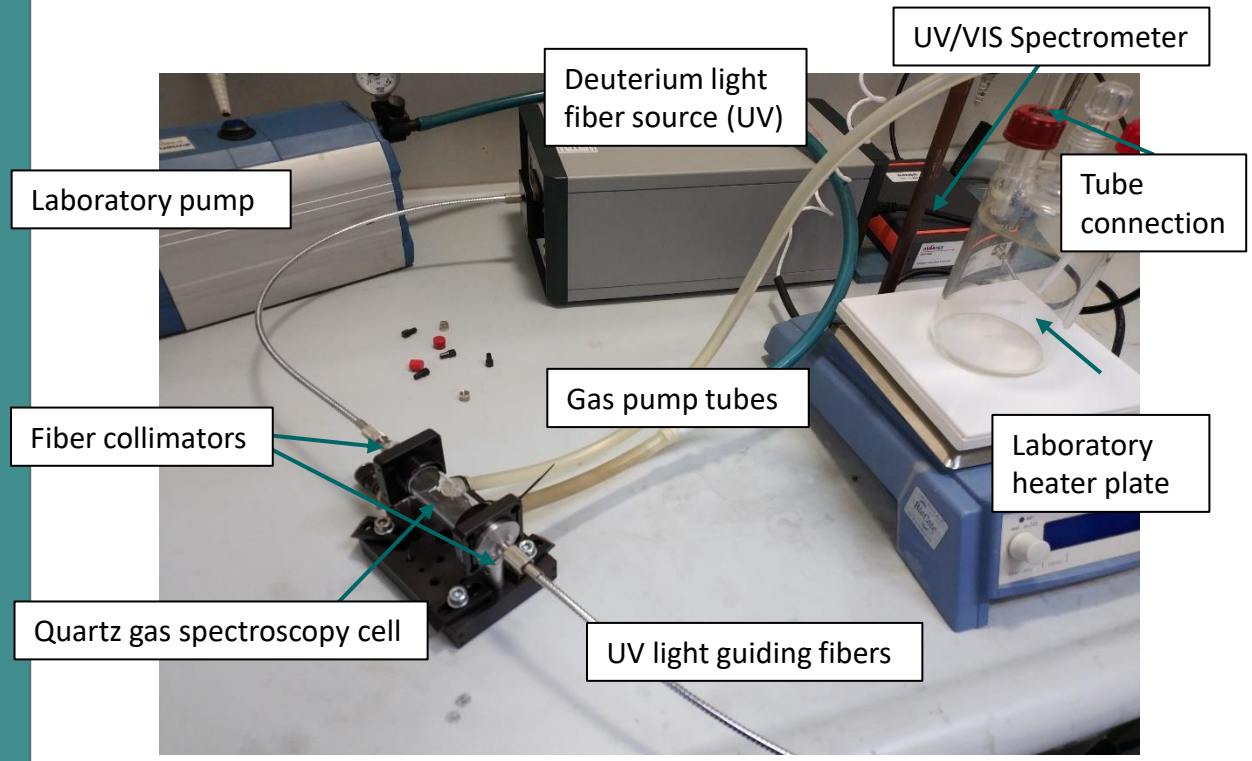




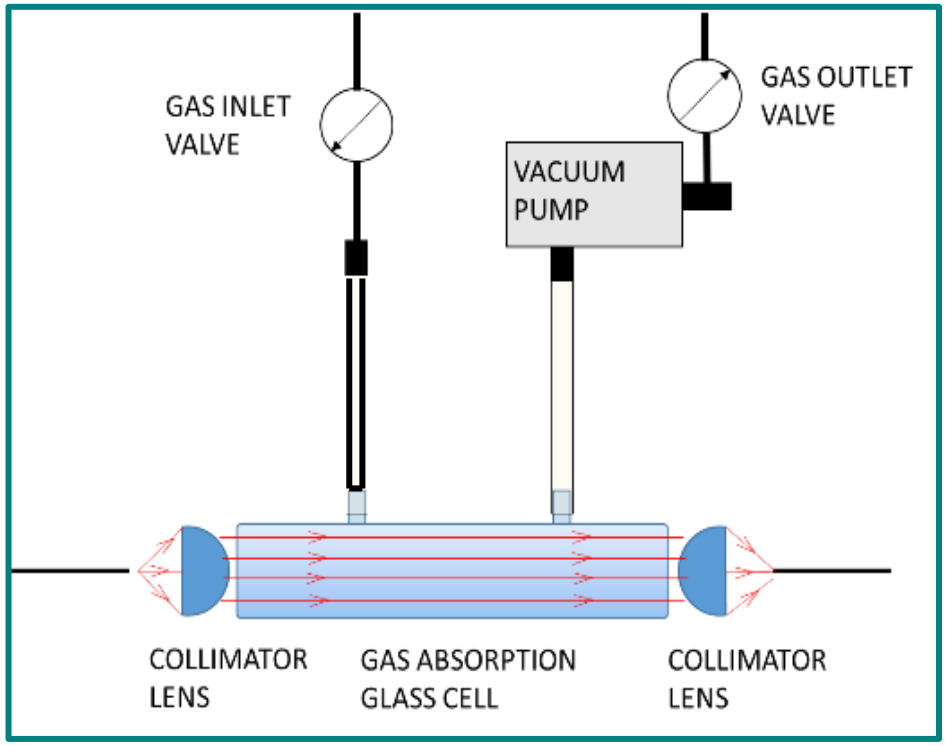


VFA sensor

VFA SENSOR
- Gas phase laboratory prototipe designed and built.
- Initial tests with Acetic Acid pattern samples undertaken.
- Sludge samples from AIMEN bioreactors tested.
- Cleaning issues between measurements need adressing.



VFA sensor



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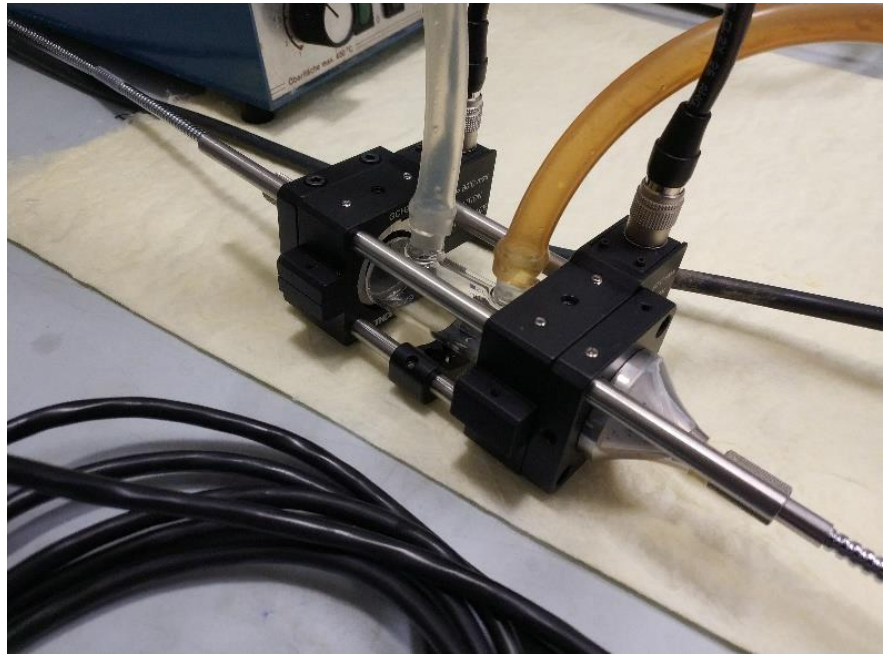
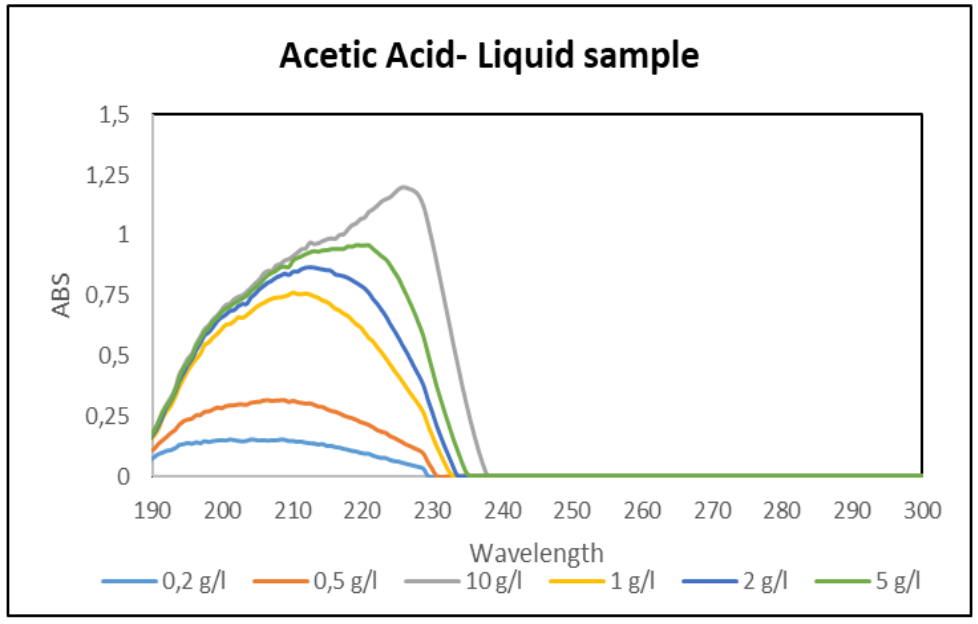
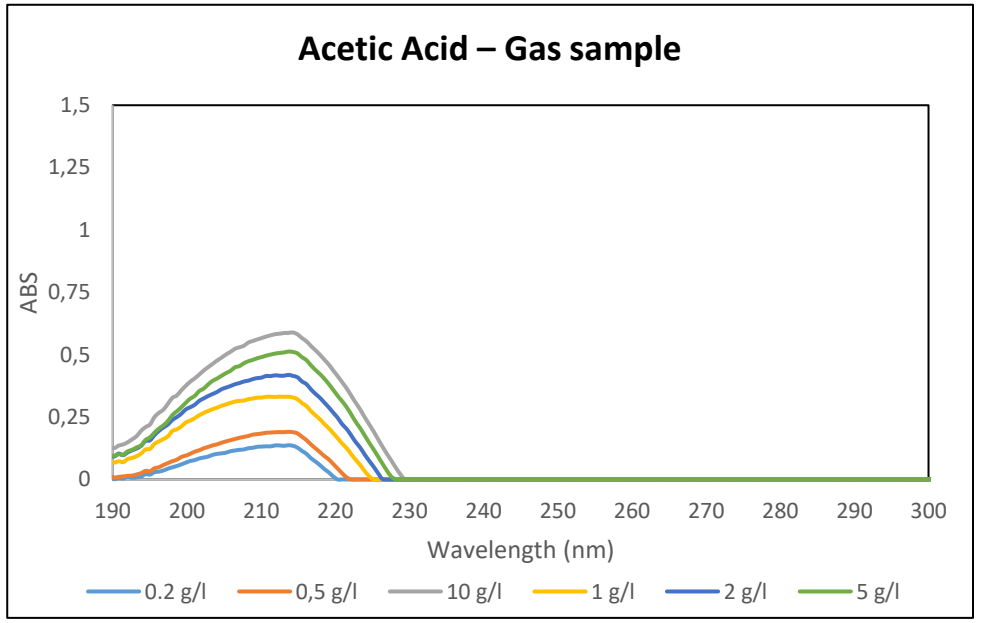


VFA sensor

Initial Tests with liquid and gas sample: Patterns

- Patterns of Acetic, propionic and butyric acid: 0.2 – 10 g /L
- Signal increment– **concentration increase.**
- Absorption peak: **220 -240 nm** for liquid sample and **210 -220** gas sample.
- Liquid phase higher signal values – more interferences
- Gas phase required exhaustive cleaning operation



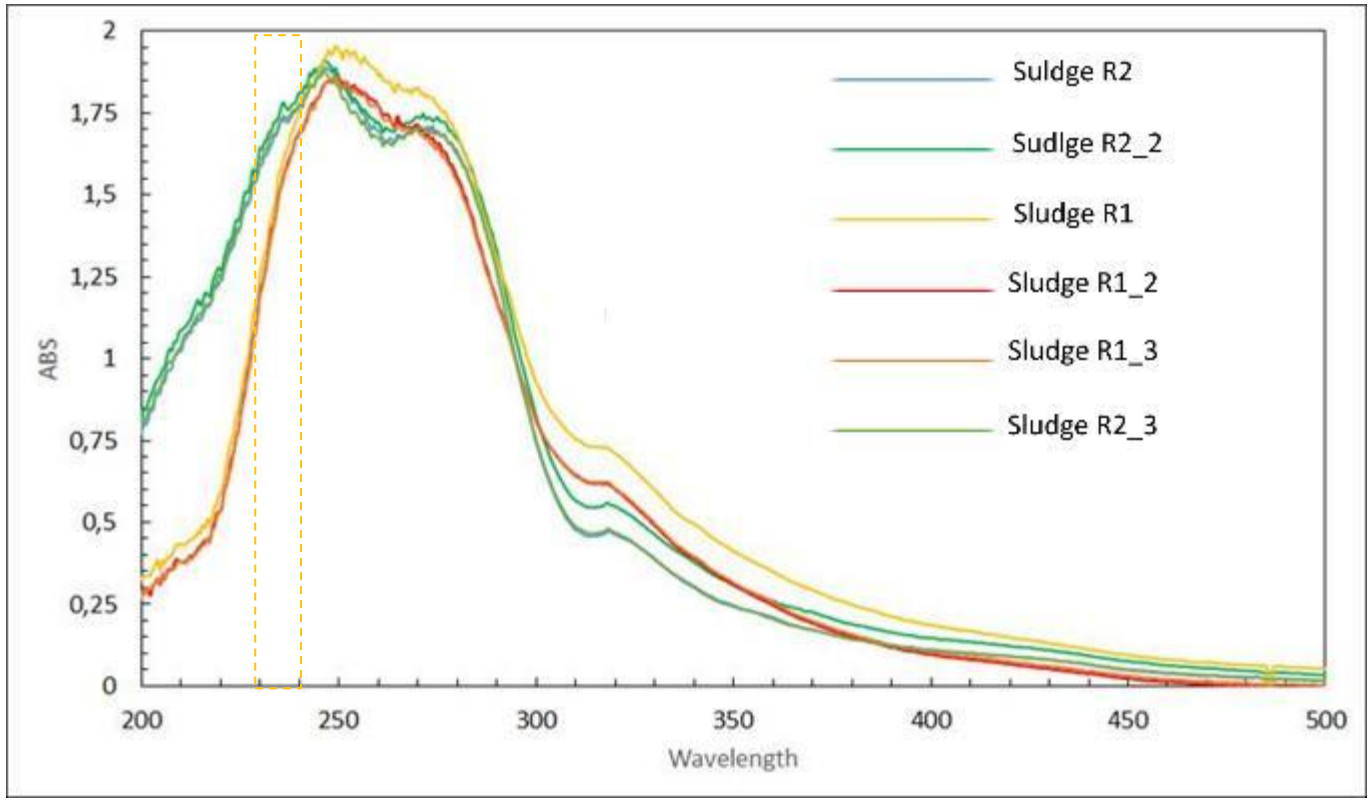


VFA sensor

Sludge from AD reactors: Liquid phase samples

- Samples from: Two lab-scale AD reactors (R1, R2 - 15 L each), stable conditions and fed with different feedstock (dairy, WWTP sludge)
- Sludge pre-treatment required - Filtration (0.45 µm)
- At 230 – 240 nm→ Sensors detected similar concentration VFA and low fluctuations
- Interference observed

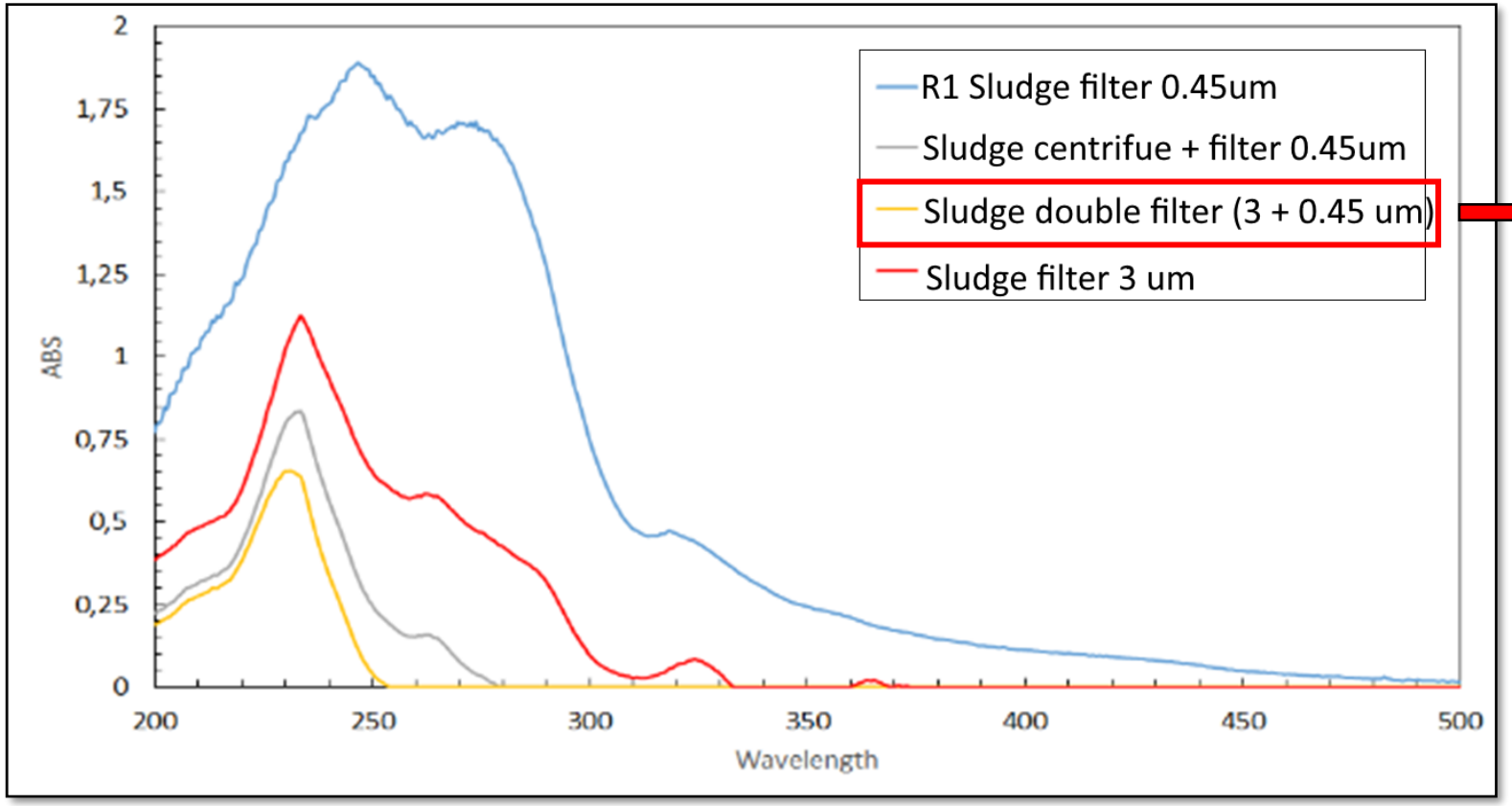
Sample	Total VFA ($gCOD_{VFA}/L$)
Sludge R1_1	0,75
Sludge R1_2	0,71
Sludge R1_3	0,73
Sludge R2_1	0,79
Sludge R2_2	1,03
Sludge R2_3	1,16



VFA sensor

Sludge form AD pilot reactors: Preparation of the samples

- Peaks not defined - Interference detected in sludge
- Sludge preparation adjusted:
  - Filtering -> simple and double filter
  - Centrifuge -> supernatant recuperation

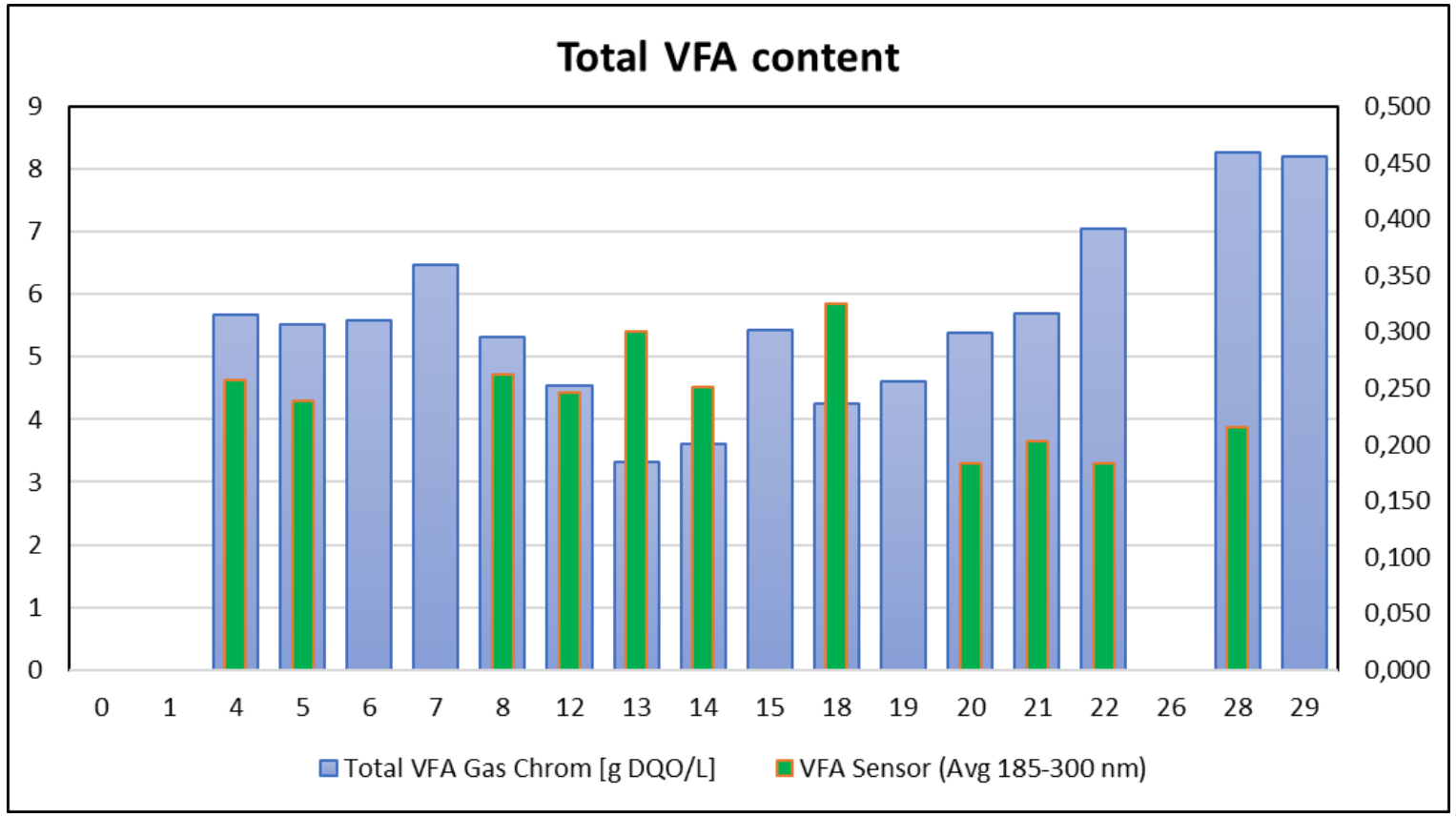


- **Double filter as best option.**
- Variability of sludge implies different optimal treatment

VFA sensor

Sludge form AD pilot reactors: Validation campaign

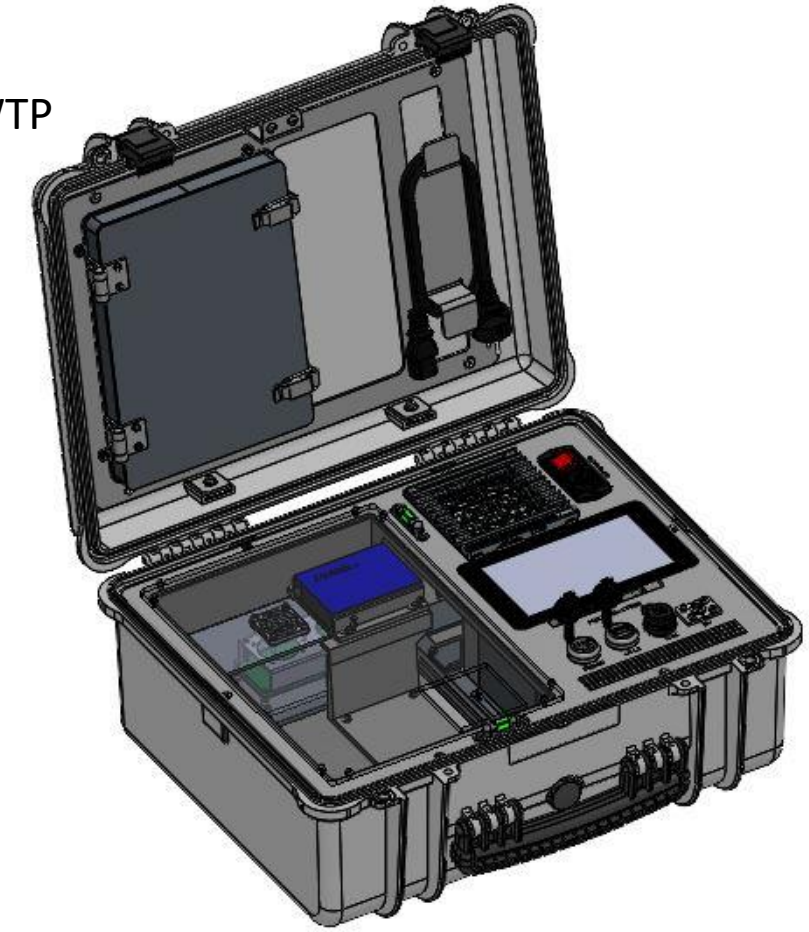
- Measurements performed during one month
- Sludge preparation by double filtration
- Total VFA checked by Gas Chromatography

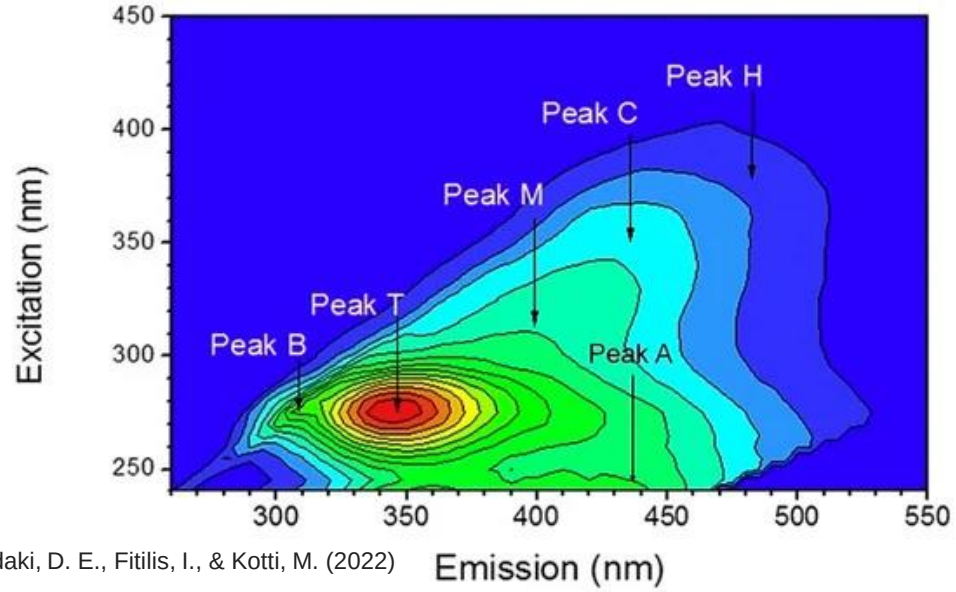


VFA sensor

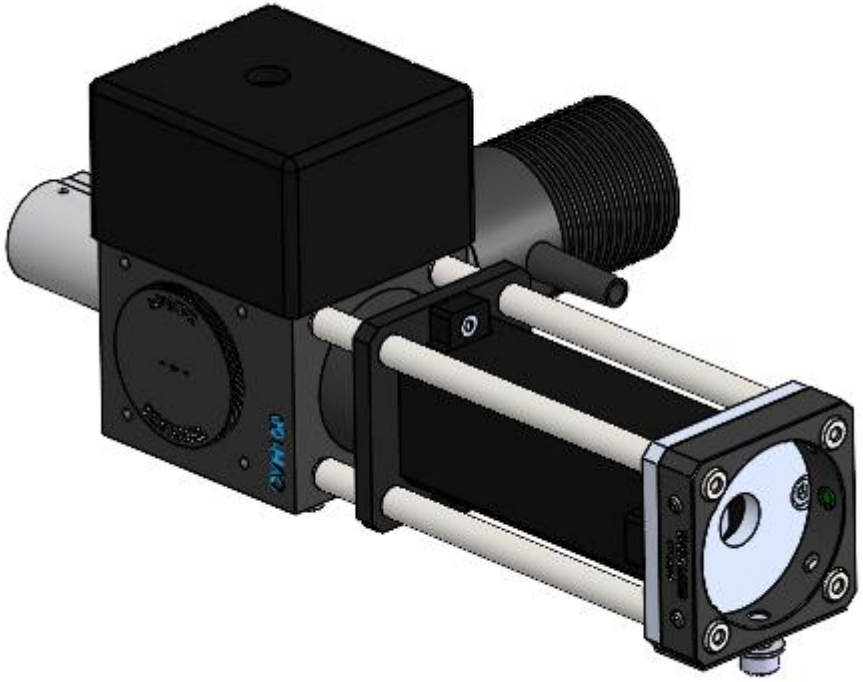
VFA sensor adaptation for PAVITR scenario

- Optics adaptation for a portable sensor → VFA sensor assembling in a portable suitcase
- Sample pre-treatment protocol and mid-term validation
- Next step: Calibration and validation in real scenario: sludge from WWTP





Zacharioudaki, D. E., Fitilis, I., & Kotti, M. (2022)

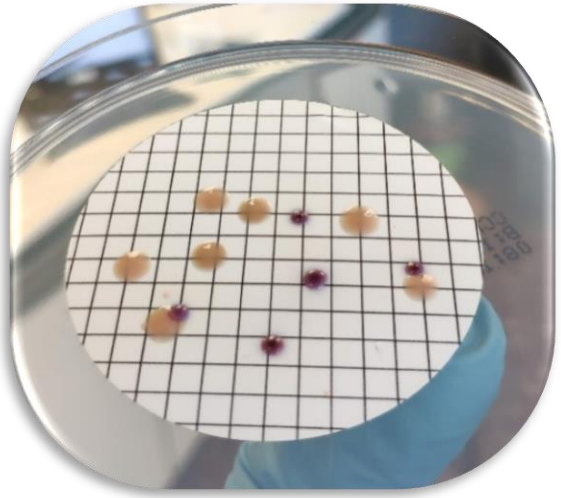


PATHOGEN SENSOR

Pathogen Sensor

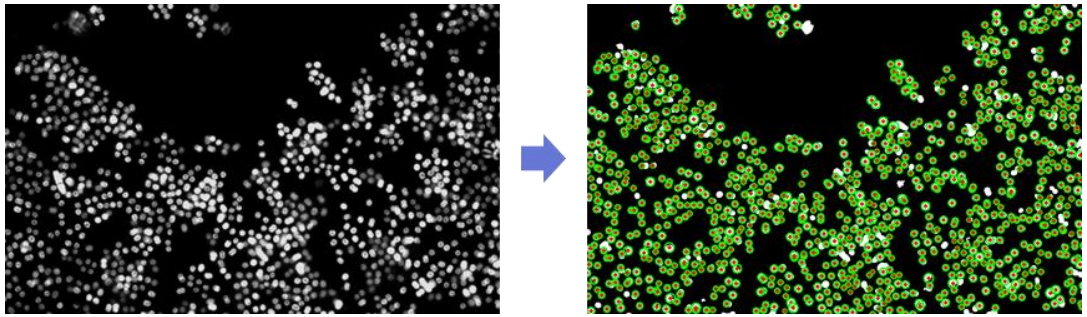
- **Sensor requeriments:** for water quality control, **monitoring the presence of *Escherichia coli* in disinfected water**
- The **pathogens sensor** will be designed to:
  - Reduce laboratory use - Field application → Portable sensor
  - Provide an easy-to-use system
  - Cost-effective materials and components.
- The sensor will determine the **presence/absence of colony-forming unit** (cfu) of *E.coli* in the volume of water.
  - A calibration and validation→ **estimation of concentration**

Image by DCStudio on Freepik

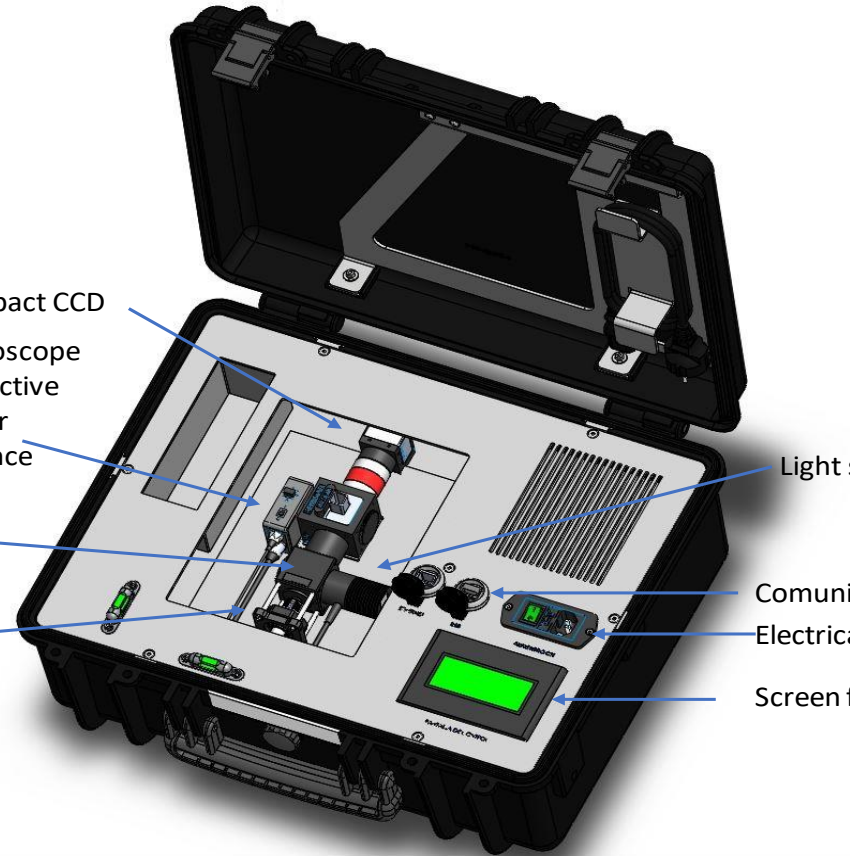


Pathogen Sensor

Based in the same portable principle as VFA sensor, in this case a fluorescence spectroscopy optical sensor will be integrated in a suitcase. The possibility of including a vision system for pathogen counting was also contemplated.



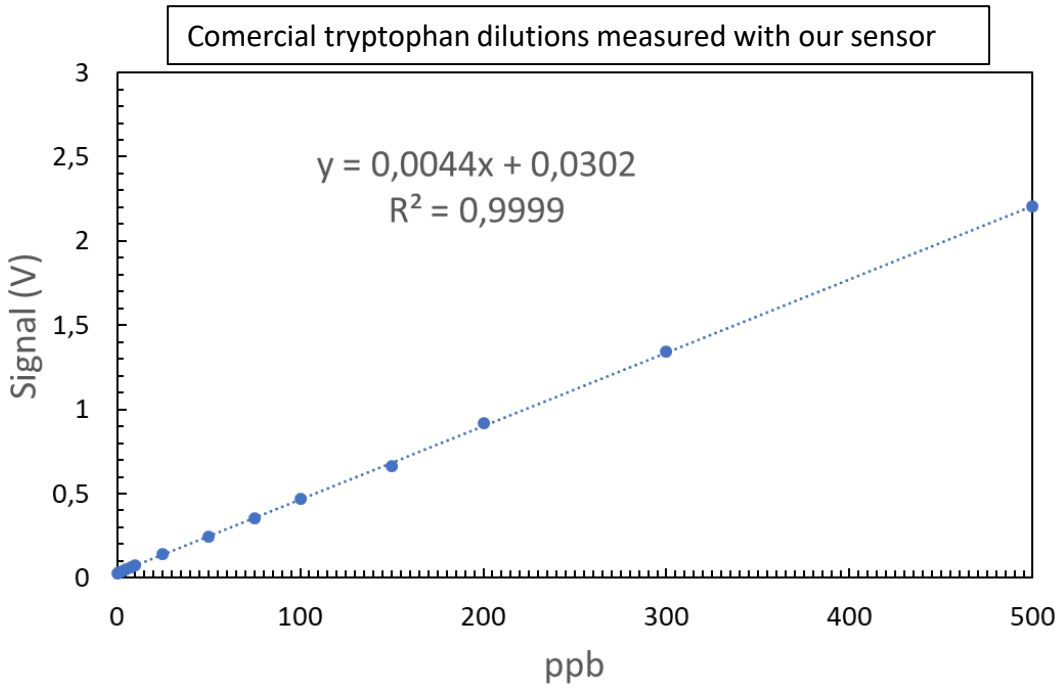
Optical sensor based on fluorescence. Main goal is to detect and quantify E-Coli by its tryptophan-like fluorescence (TLF) properties.

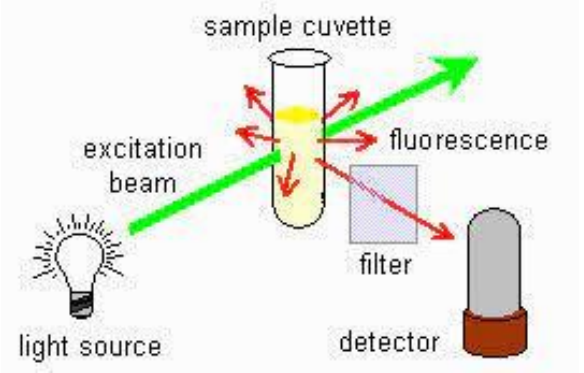


AIMEN is already experienced in integrating portable sensors for field measurements which combine roughness, precision and flexibility in data collection and transmission.

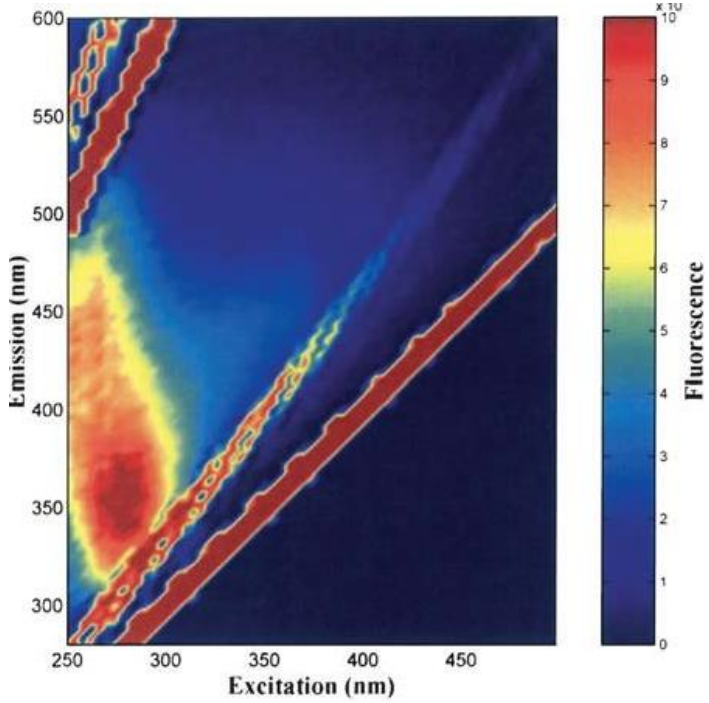
Pathogen Sensor

- The sensor working principle is based on the tryptophan content in the *E. coli* which presents **fluorescence response**.
- The optimal wavelengths for measuring tryptophane-like material: **Ex 280 nm - Em 340nm**





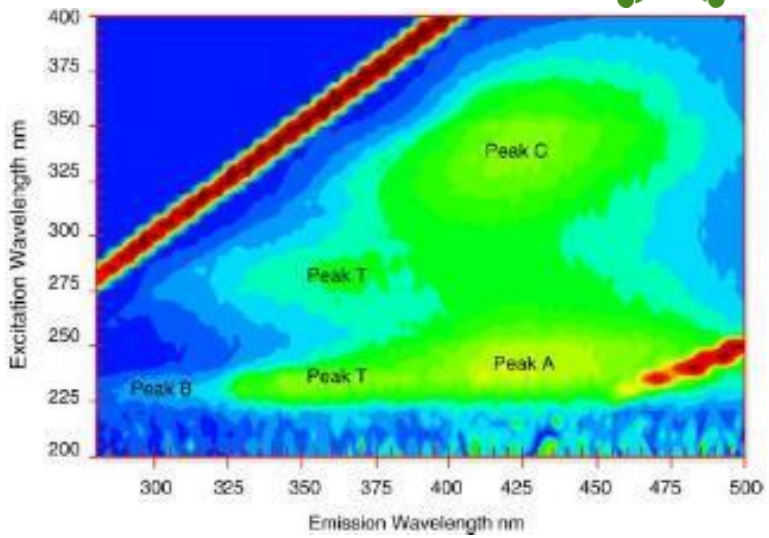
Direct correlation observed between tryptophan concentration and signal detected by the sensor

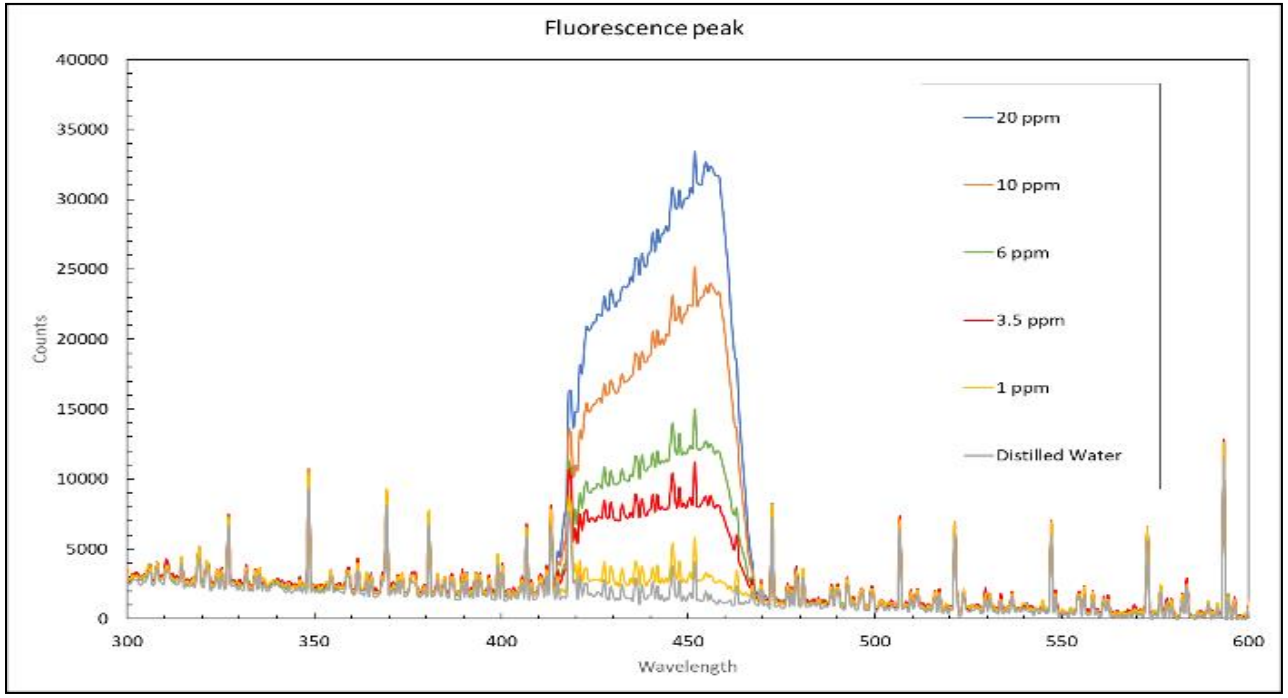


EEM – Tryptophan like compounds
Source: Kowalczuk, P., et al. (2003)

Pathogen Sensor

- Working principle: Fluorescence of tryptophan content in the *E. coli.*
- **Fluorescence-based** sensor
- Measurements performed with other instruments for fluorescence performance of the samples
  - ✓ Laboratory fluorimeter with colormap graph (emission and absorption peaks detected)
  - ✓ Fibered spectrometer (same as used in VFA case) for fluorescence peak detection.
  - ✓ Photomultiplier (PMT) The one finally selected for PAVITR sensor development.



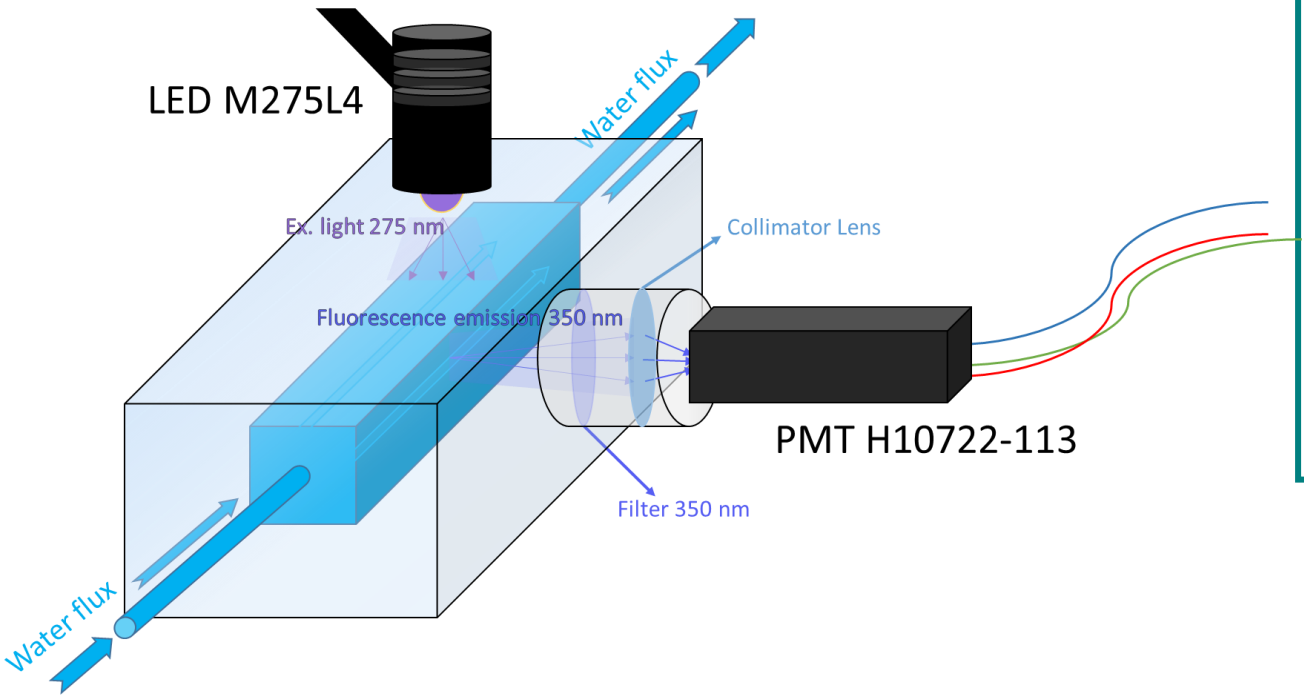


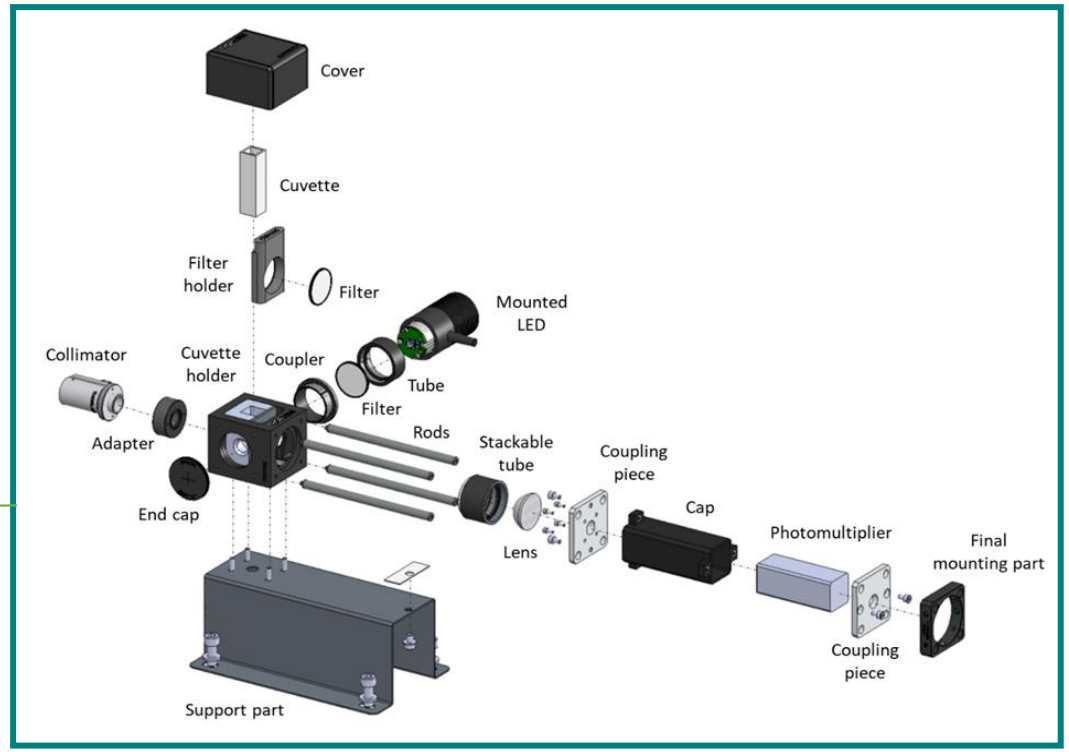
Pathogen Sensor

PAVITR

PATHOGEN SENSOR

- Laboratory setup with Photomultiplier (PMT) already designed, built and tested.
- Two prototipes tested, one with peristaltic pumping of the sample using flow through cuvette, and a static one.



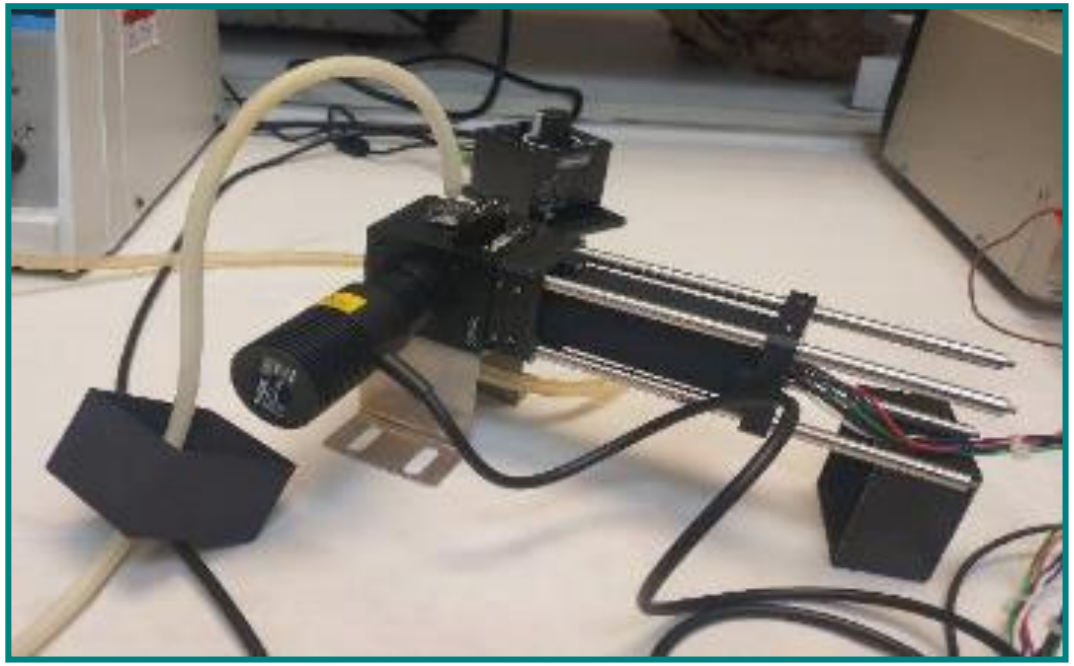


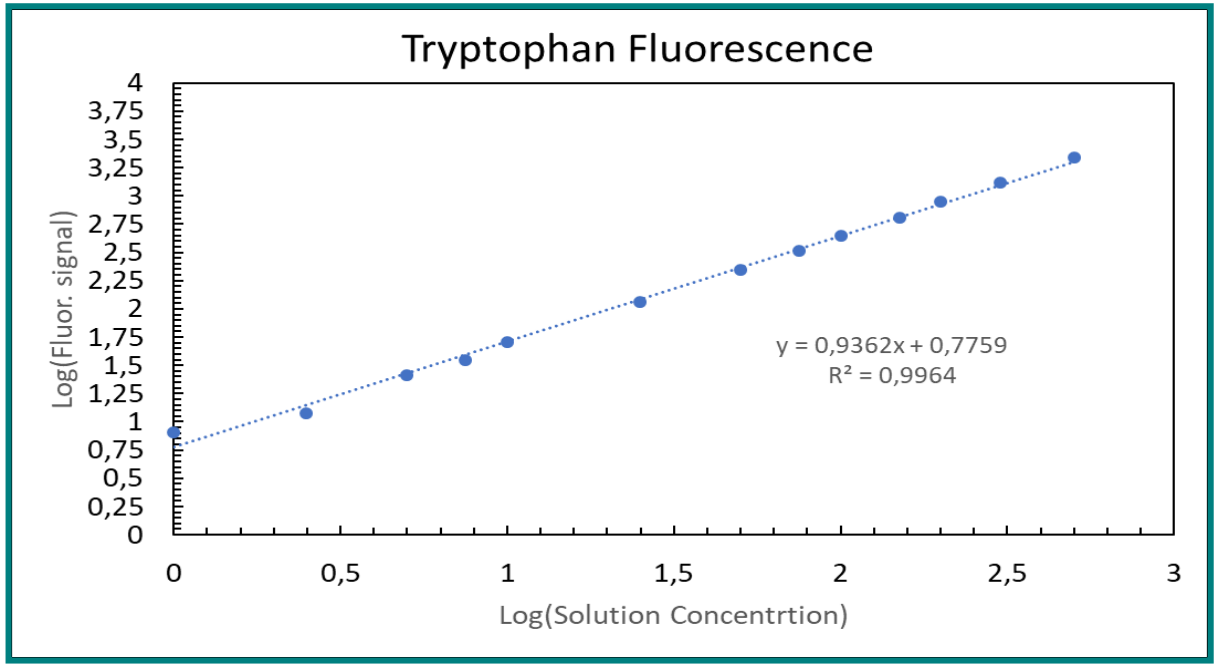
Pathogen Sensor

PAVITR

Fluorescence-based sensor for *E.coli* monitoring in drinking water - Initial tests

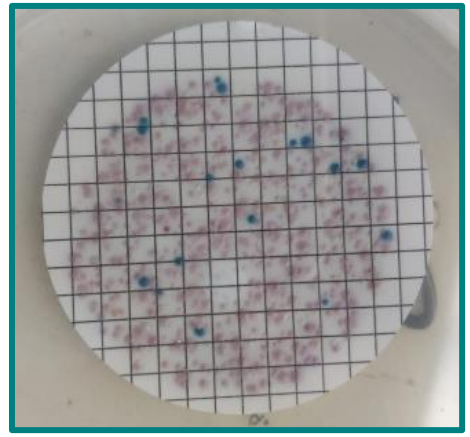
- Pattern samples -> tryptophan (1 - 500 ppb in distilled water)
- Direct correlation – Tryptophan -Signal
- Optimal wavelengths: **Ex at 280 nm and – Em 340nm** (Tryptophane-like material)



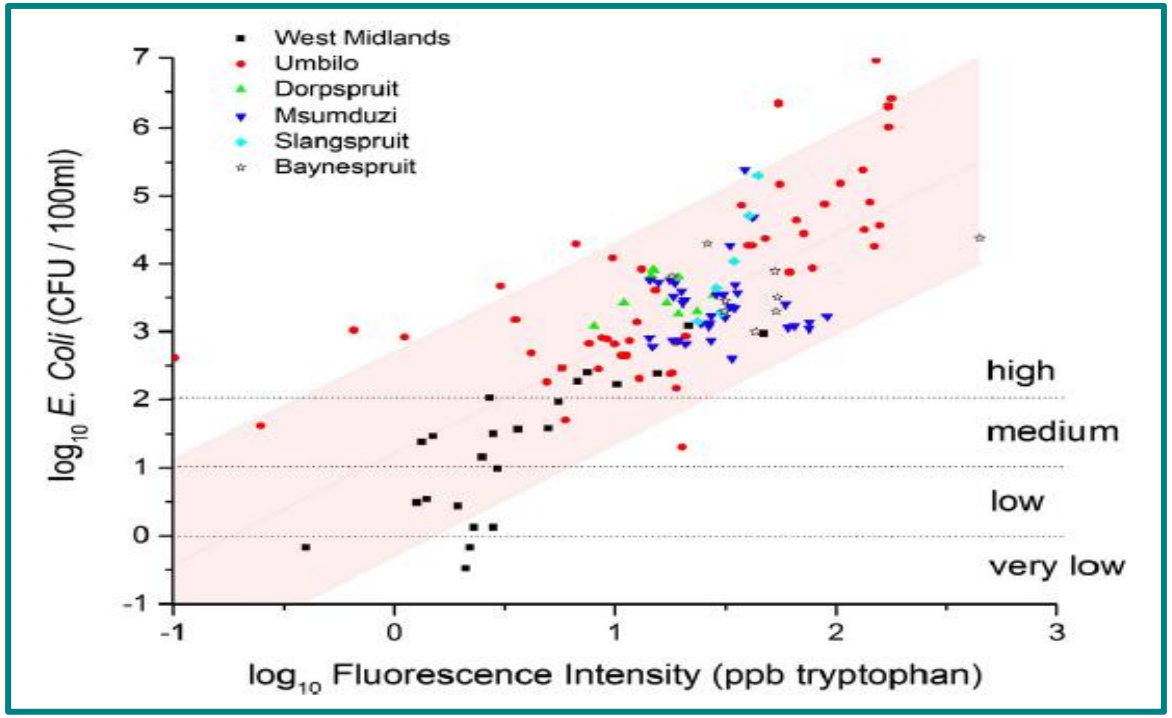


Pathogen Sensor

- E. coli culture used to pollute distilled water
  - *E. coli* (ATCC® 8739) was grown in LB-broth media
  - Phosphate Buffered Solution for removing the LB broth -> interference in fluorescence
  - **Additions (dilutions) in distilled water** as pollution simulation
- Quantitative experiments to determine the capacities of the sensors.
- Measurement validated using agar plate method (ISO 9308-1)



Results obtained must match the data from bibliography.



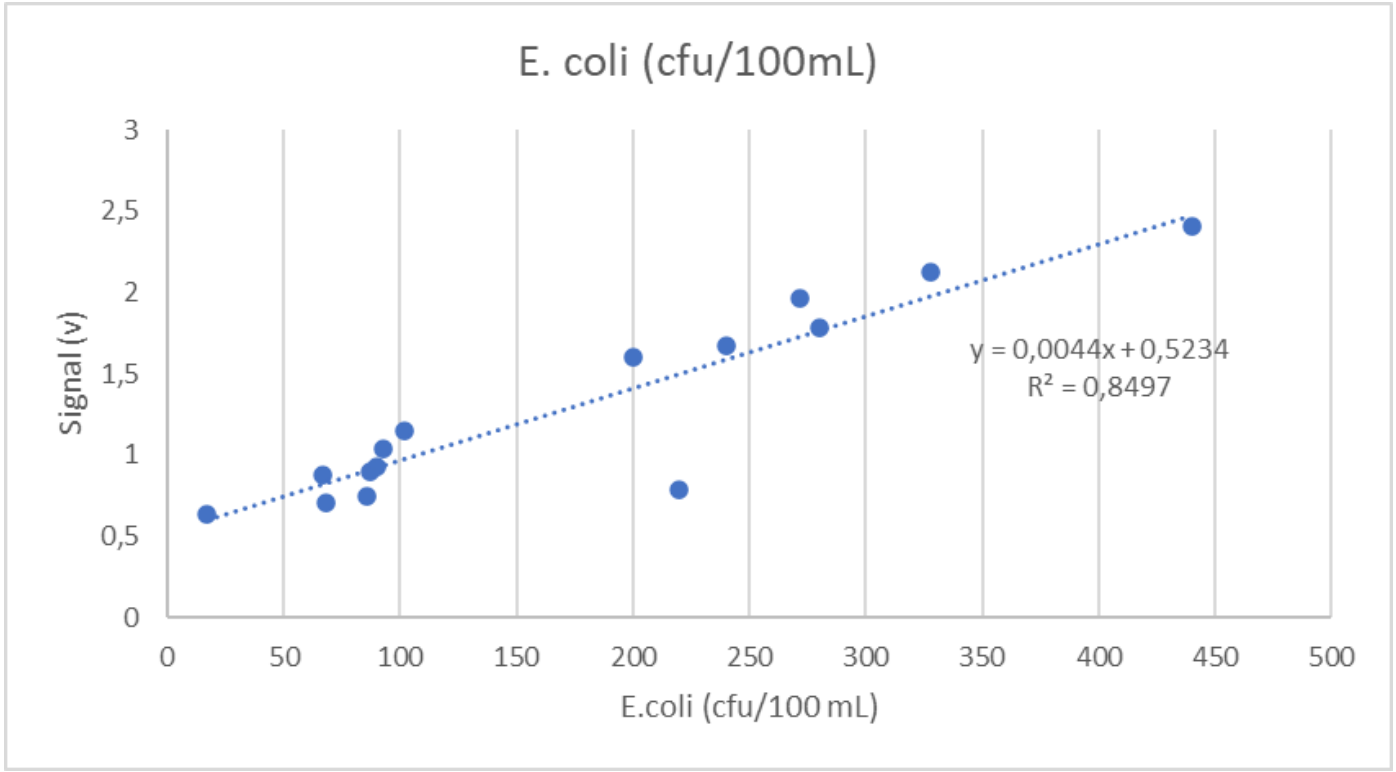
Pathogen Sensor

PAVITR

Fluorescence-based sensor - *E.coli* laboratory assays

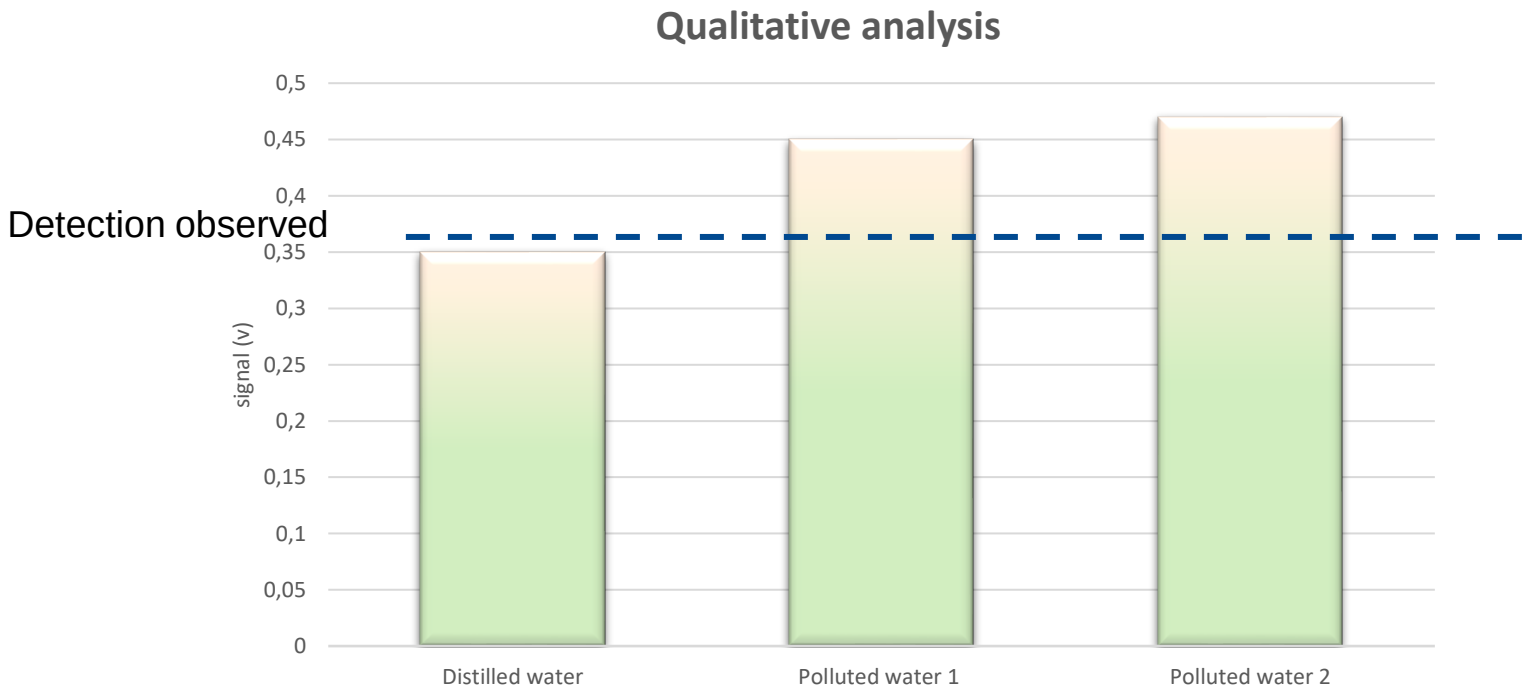
- *E.coli* broth used to pollute distilled water
- Quantitative experiments to determine the capacities of the sensors .

E. coli (cfu/100mL)	Signal (V)
17	0,64
68	0,71
220	0,79
86	0,75
67	0,88
87	0,9
90	0,93
93	1,04
102	1,15
200	1,6
240	1,67
280	1,78
272	1,96
328	2,13
440	2,41



Pathogen Sensor

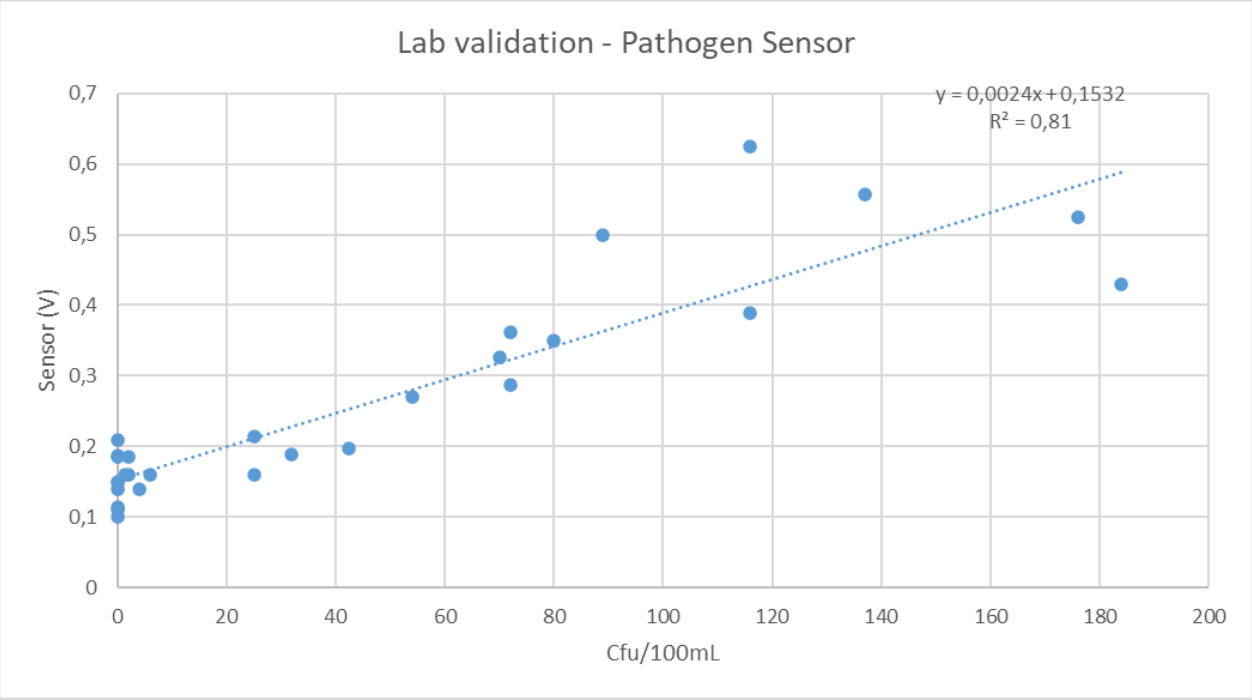
- Qualitative assays to determine sensor capacity to detect the presence of E. coli
- Real water samples diluted in distilled water → reduction of bacteria in sample.
- Detection of signal in different samples

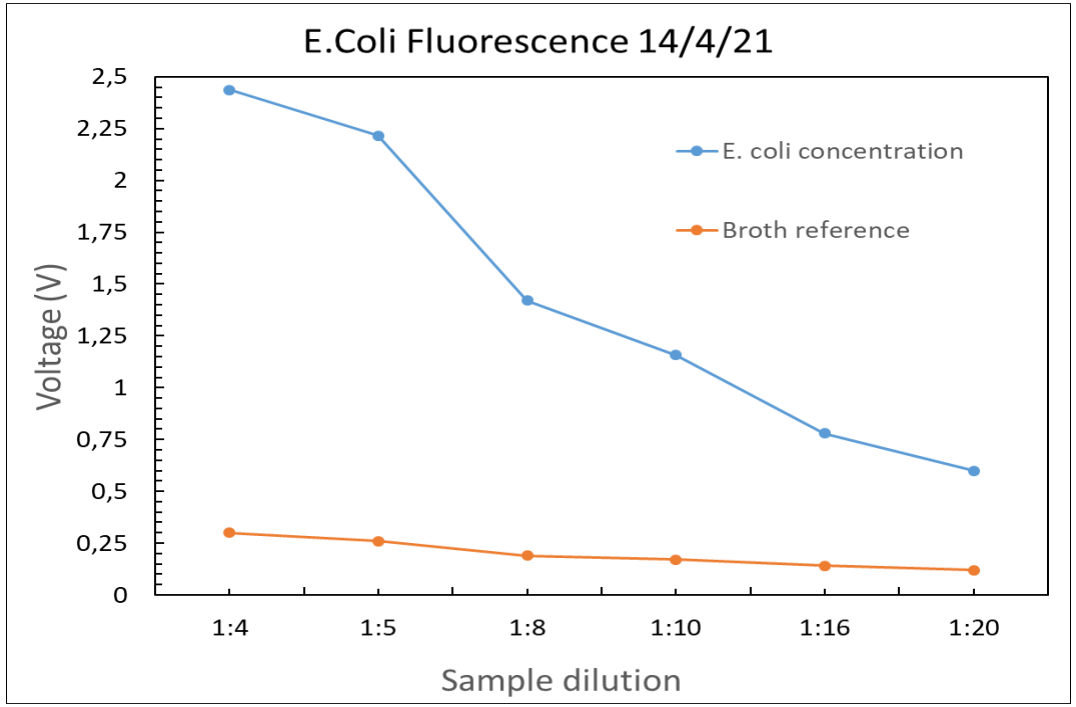


Pathogen Sensor

Fluorescence-based sensor - *E.coli* laboratory assays

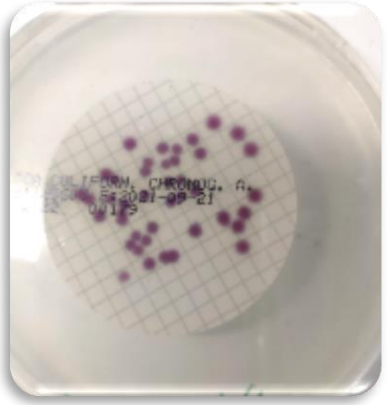
- Second set of Assays to determine sensitive of the sensor working with polluted distilled water.
- Comparison between the dilution of E. coli cultivation sample with broth and a reference broth sample in the same proportions (to discard signal contamination from possible broth fluorescence).

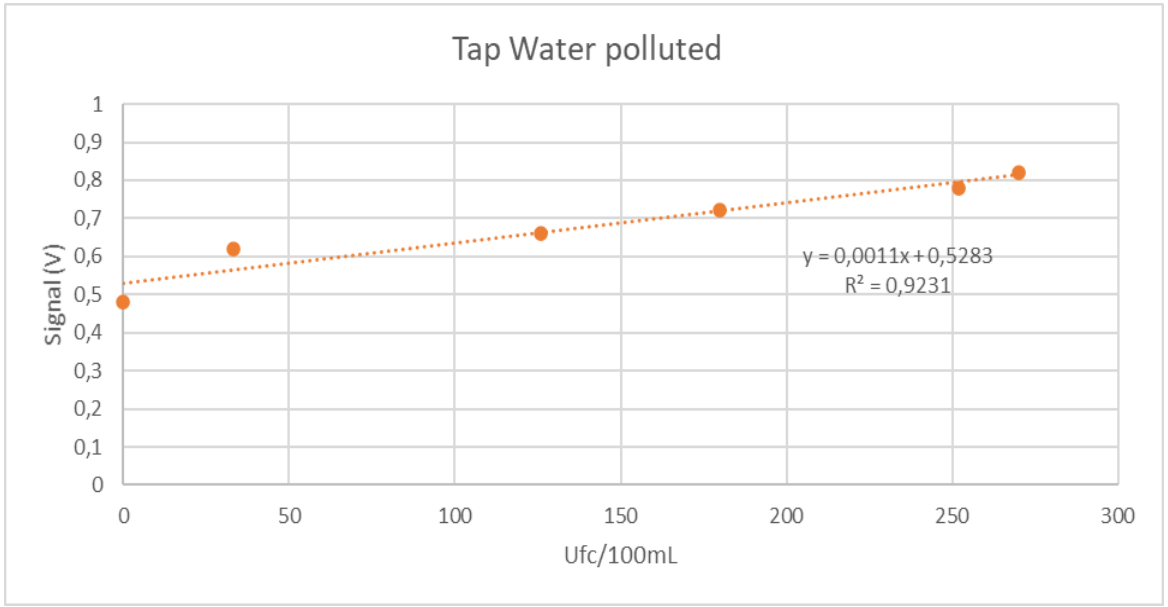




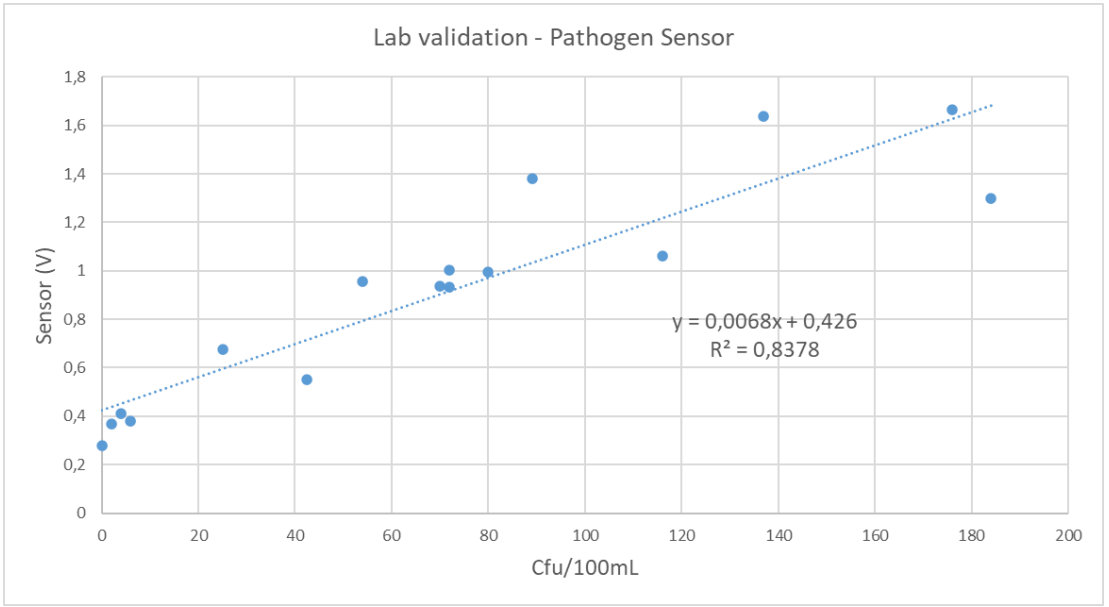
Pathogen Sensor

- **Sensitive - quantitative analysis**:
  - *E. coli* culture used to pollute distilled and tap water (*E. coli* (ATCC® 8739))
  - Measurement validated using agar plate method (ISO 9308-1)
- **Final validation in lab conditions: Distilled and Tap water**
  - Next validation should be performed in Aligarh (India)





Tap water assay



Distilled water tests

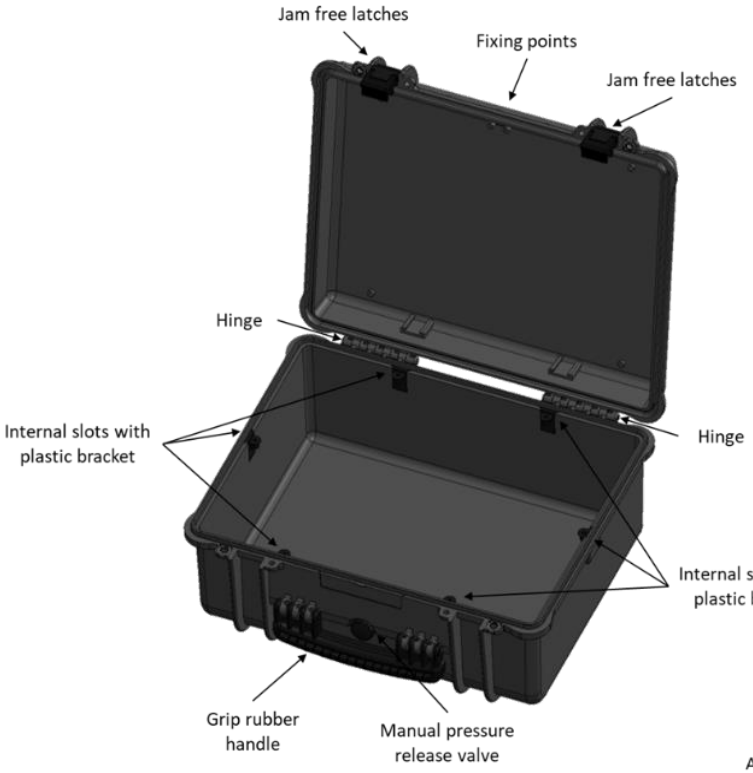
Let’s have a break

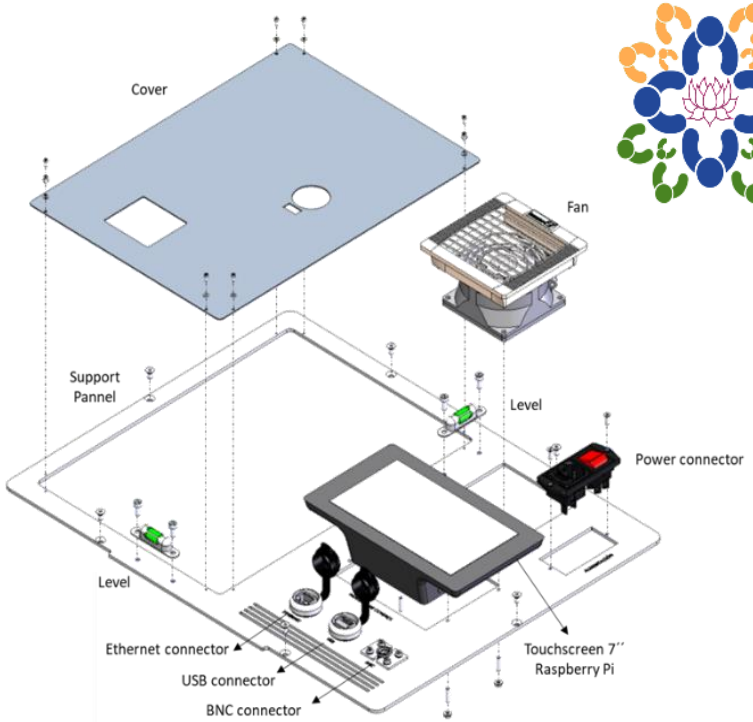
We will be back in 30 min

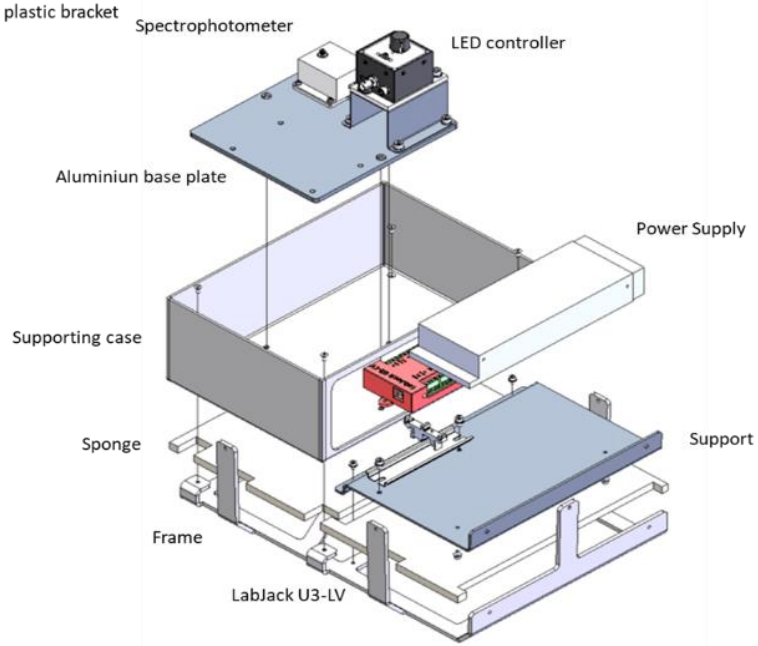
Assembly and implementation

Sensors’ suitcase assembly and components

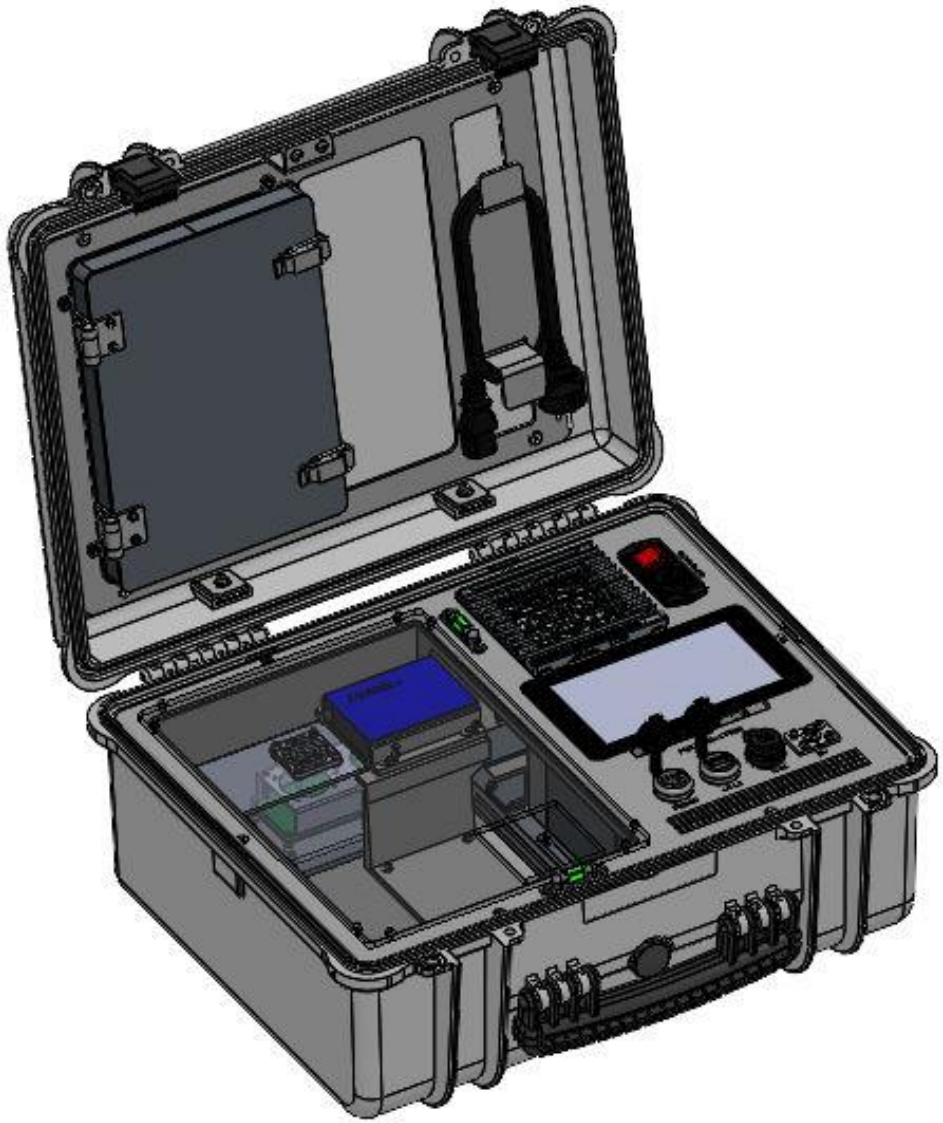
Sensors' suitcase design







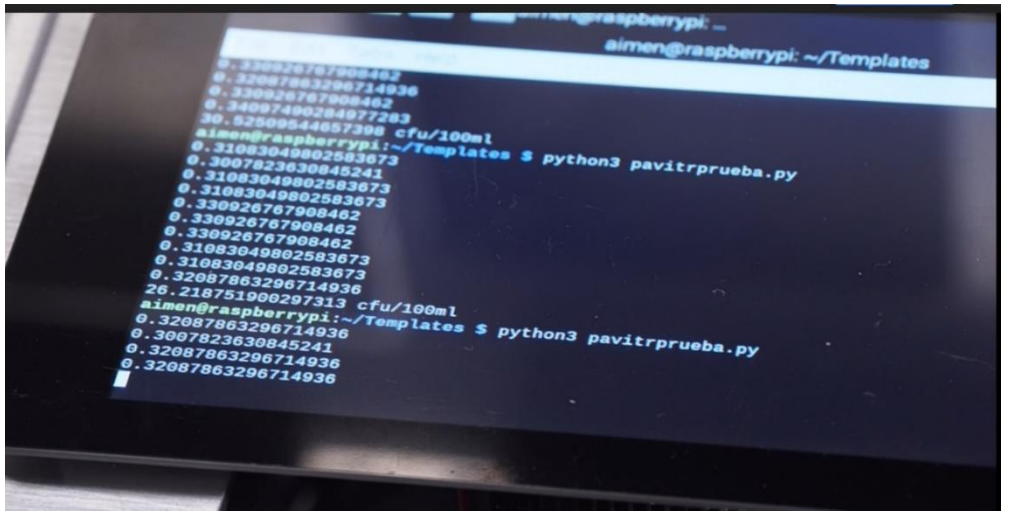
Both sensors have the same structural design, same batteries, Raspberry and touchscreen display, electrical connections and plugs. Only sensing devices and light sources (and their electronic control) are different.



VFA SENSOR

VFA Sensor

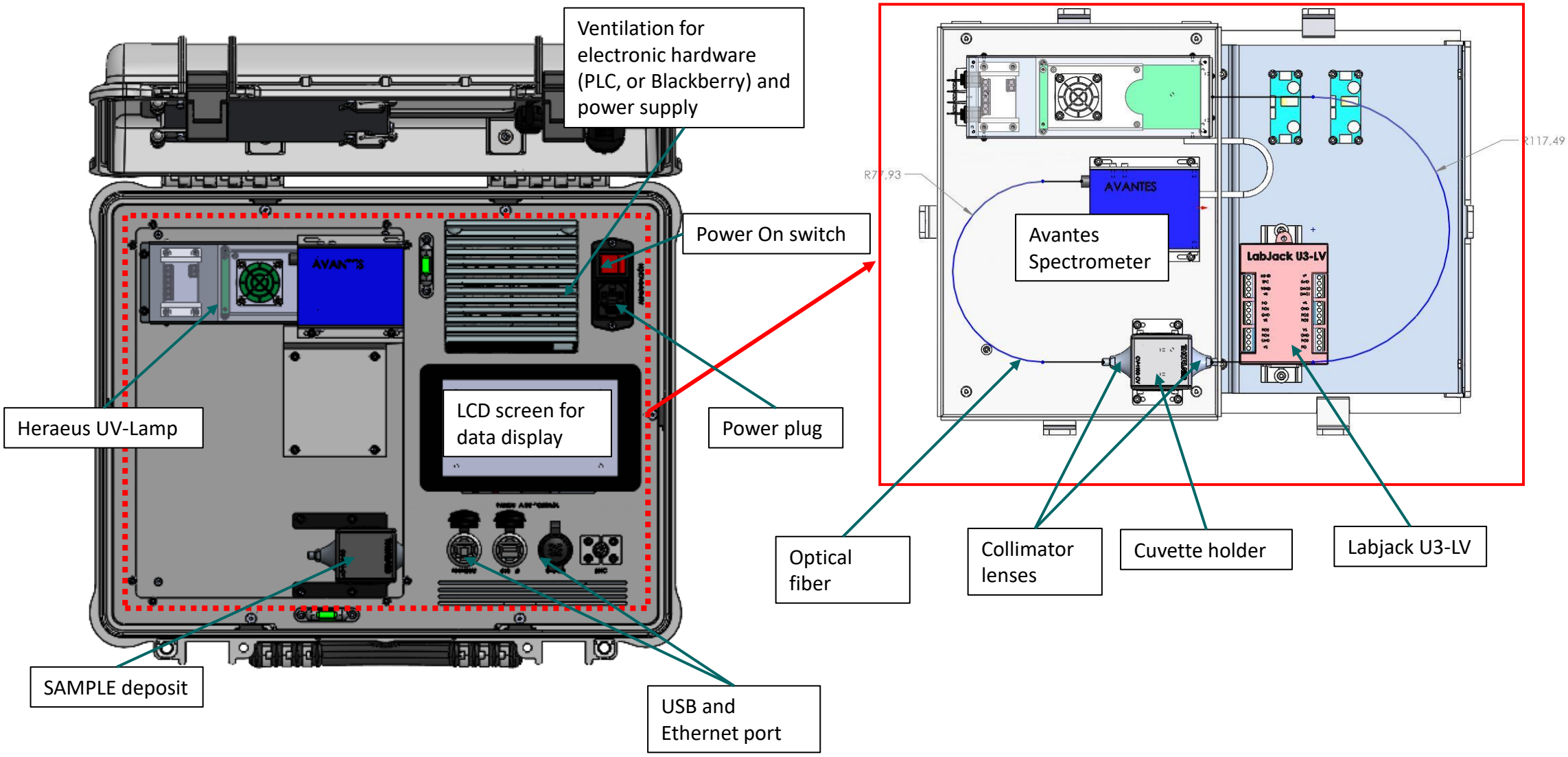
- Set up to suitcase → Structural elements attached to the suitcase. Assembly of the optical VFA sensor inside (fibers, collimators, lamp, etc.). Electronic connections Raspberry-Spectrometer. Battery and electrical circuits connected.



Close-up of PAVITR sensor touchscreen for sensor control and data visualization.

Structural pieces for the suitcase sensor design made of inox steel and also 3D-printed elements for components assembly (spectrometer holder, cuvette and battery structural support, etc.)

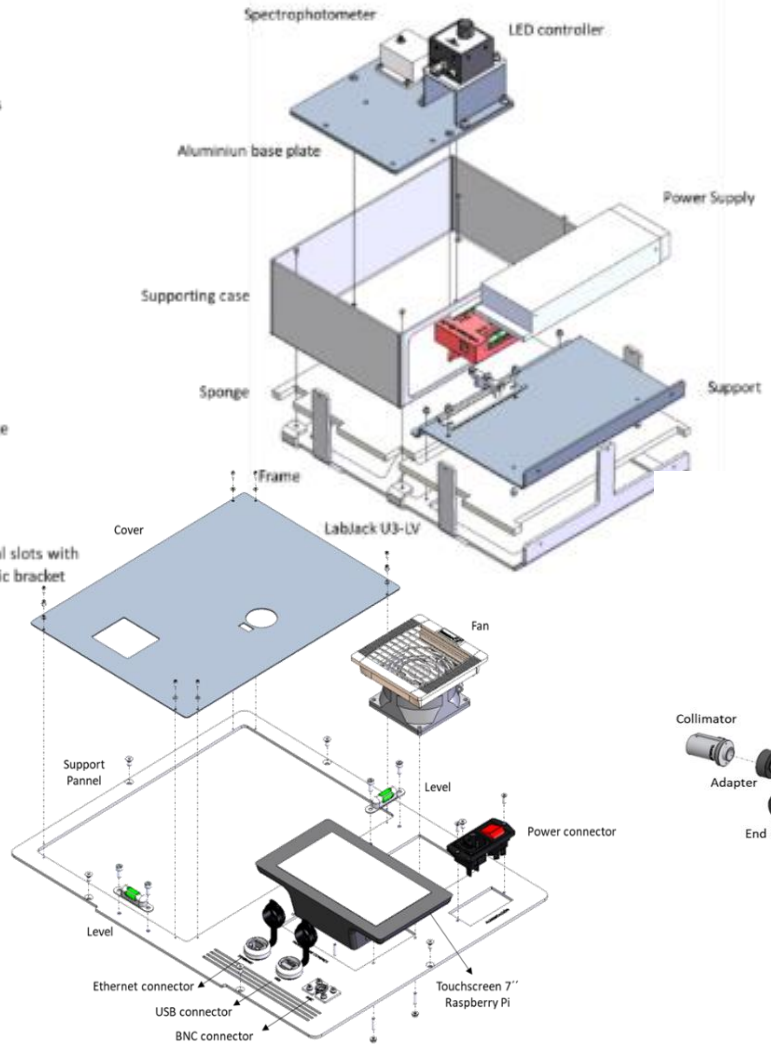
VFA Sensor

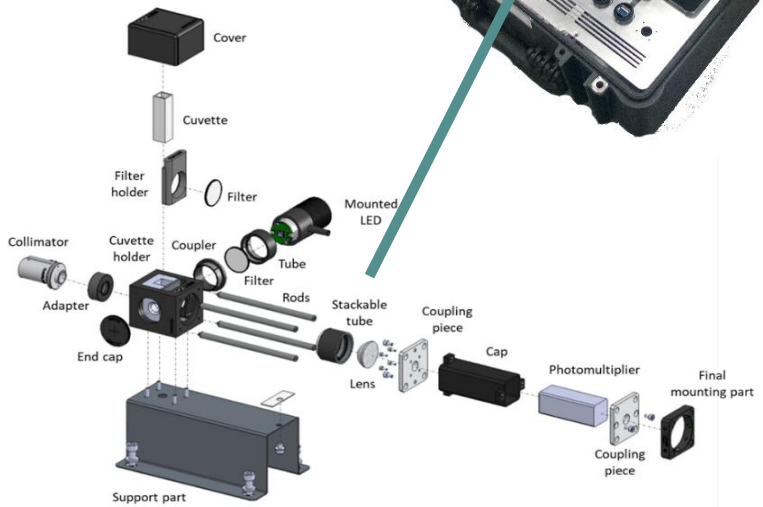

Ventilation for electronic hardware (PLC, or Blackberry) and power supply
Power On switch
Heraeus UV-Lamp
LCD screen for data display
Power plug
SAMPLE deposit
USB and Ethernet port
AVANTES
Avantes Spectrometer
LabJack U3-LV
R77.93
R117.49
Optical fiber
Collimator lenses
Cuvette holder
Labjack U3-LV

PATHOGEN SENSOR

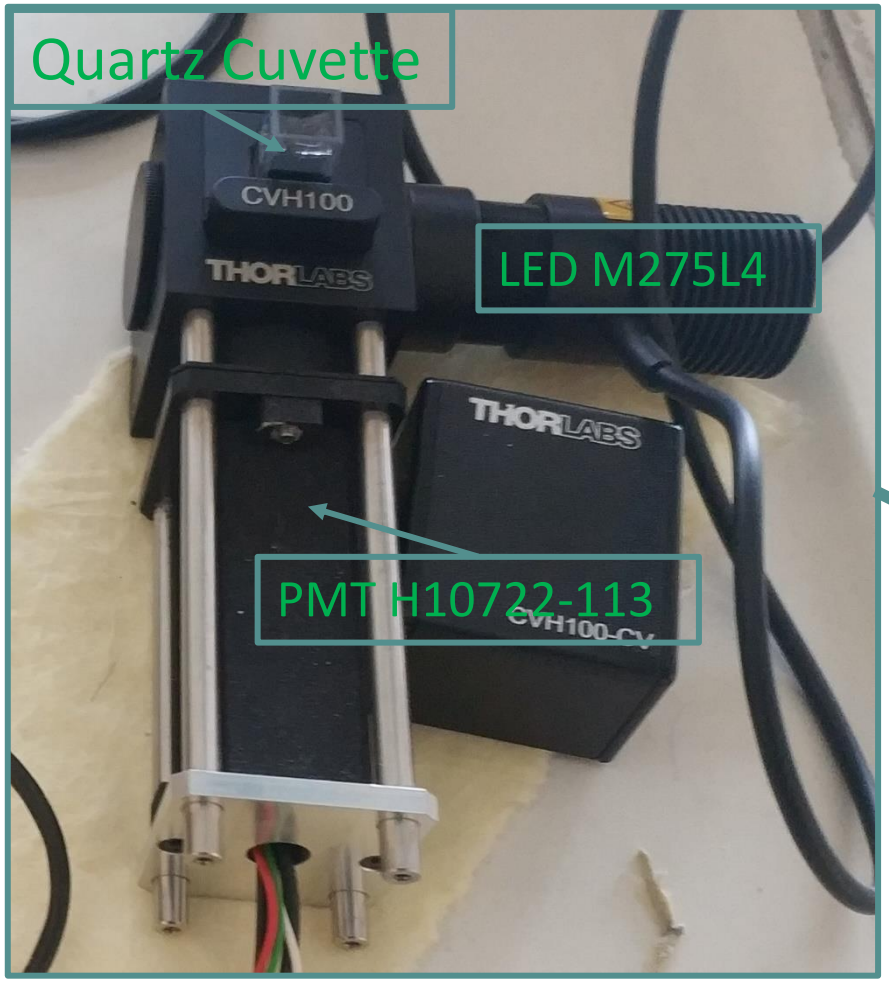
Pathogen sensor

- Set up to suitcase → Structural elements attached to the suitcase. Assembly of the optical pathogen sensor inside. Electronic connections Raspberry-Labjack. Battery and electrical circuits connected.

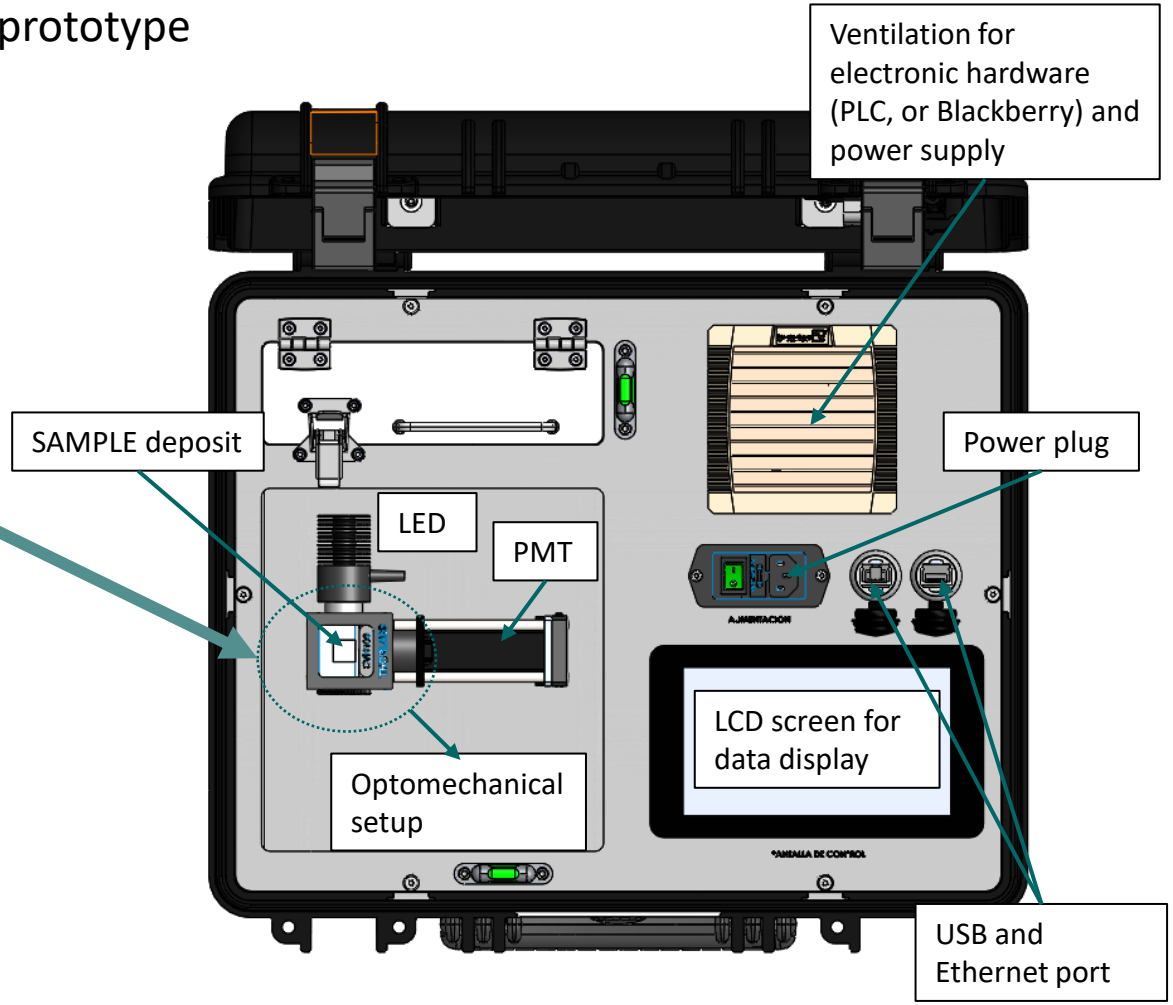




Pathogen sensor



Conceptual draft and component description for a suitcase sensor prototype



Pathogen Sensor

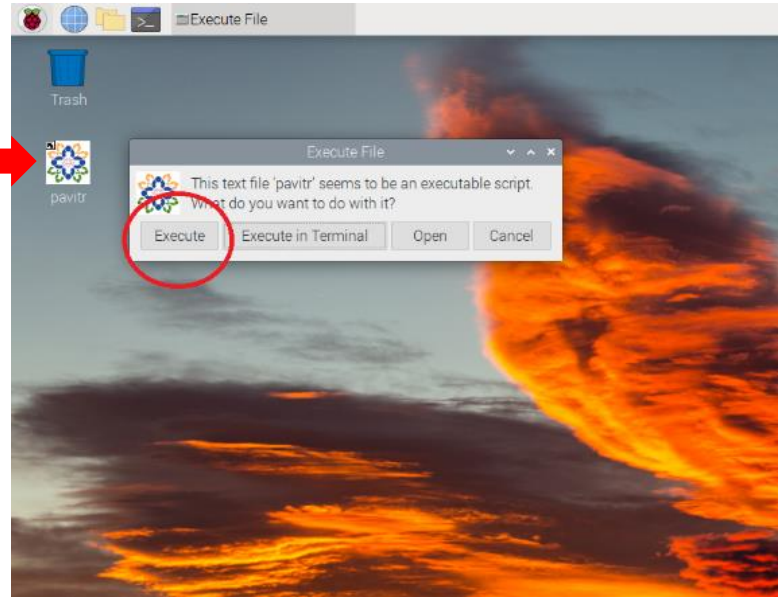
- **Optical and mechanical elements assembled** in AIMEN
  - Emission arm: LED source and bandpass (280 nm);
  - Cuvette sample holder
  - Detector arm : Collimating lens (350 nm)
  - A photomultiplier module

- **Portable sensor** for field measurements
  - Results in seconds
  - Touchscreen for sensor control and data monitor.
  - Sensor operative in Aligarh (India) since January 2023

Operation and maintenance

Sensors operation interface

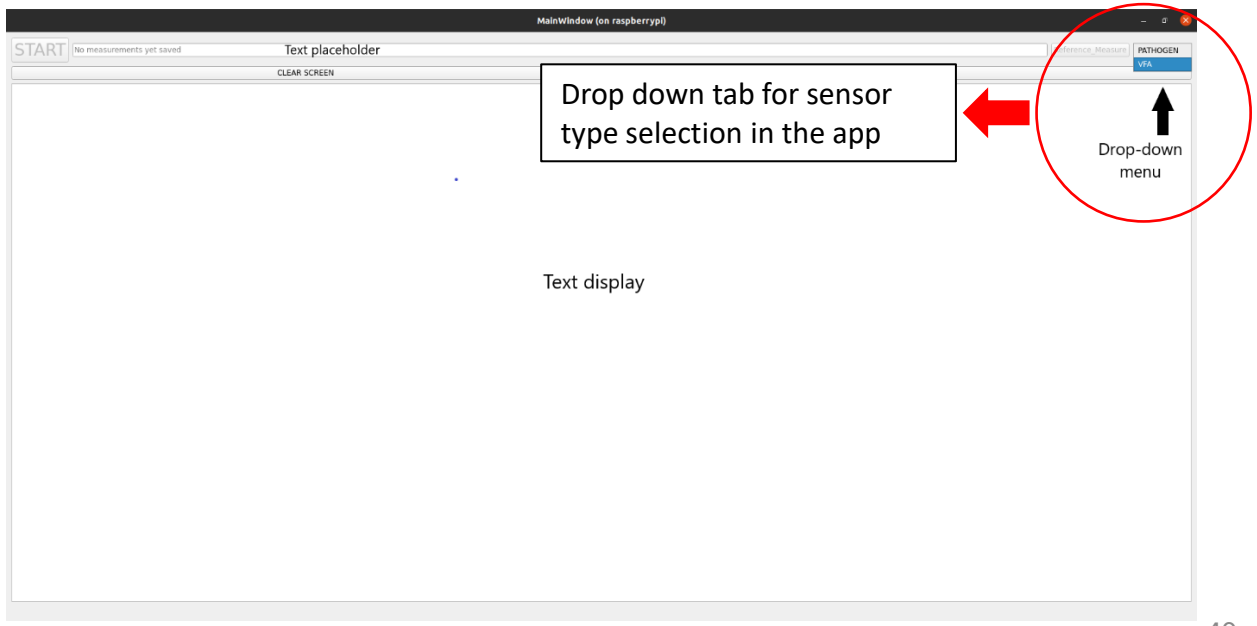
PAVITR icon on desktop



Sensors work independently and run on batteries. Batteries need to be charged through the supply cable connected to the plug, next to the switch button.

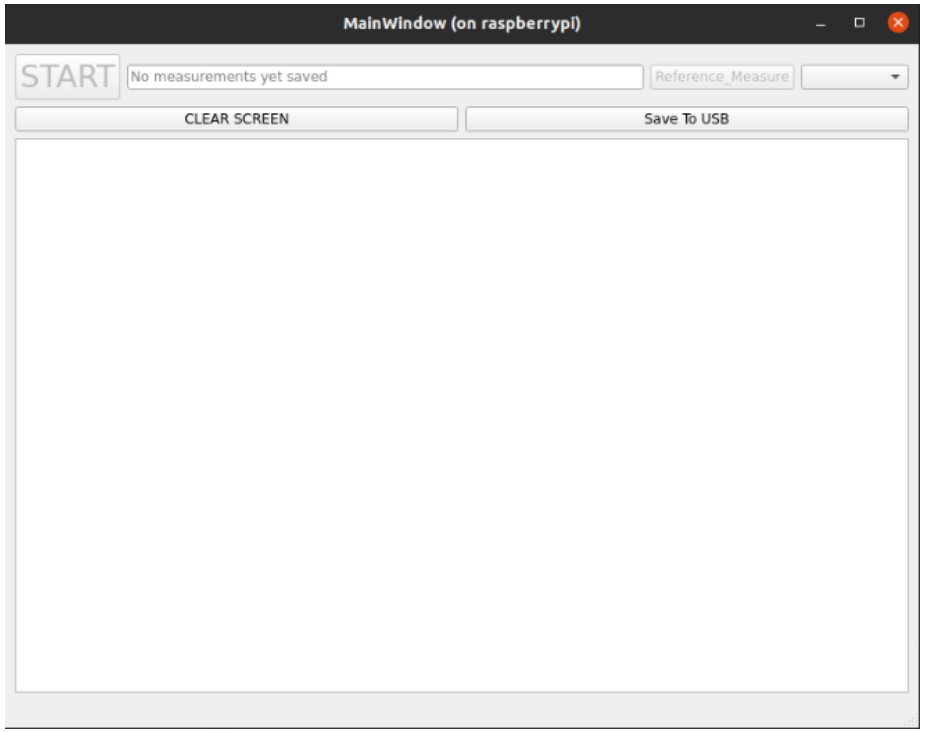
Sensors are controlled using the Raspberry Pi 7'' Touchscreen.

- Once the suitcase is open and the ON-OFF switch is on, the Raspberry will automatically start-up.
- The PAVITR application will automatically open when booting the system. However it can be opened through the PAVITR icon on the desktop.
- The same application runs for both sensors and it allows to launch the measurements and store the data. The sensor type must be selected before running the measurements.

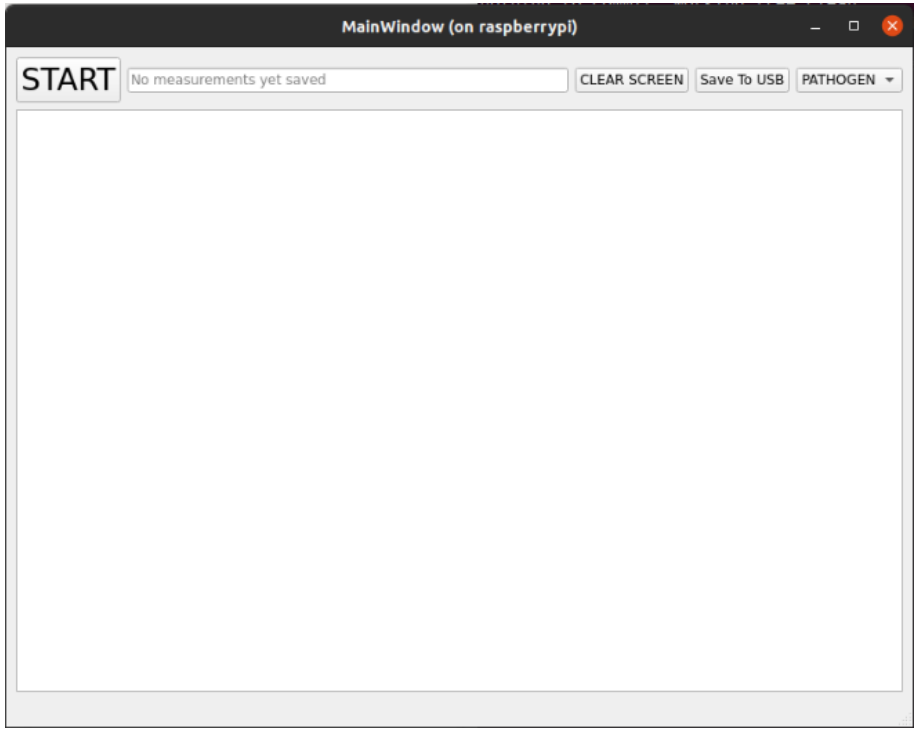


Sensors operation interface

- VFA sensor allows us to make a reference measurement (with distilled water), that will be automatically saved internally. Then the measurement will be done pressing the START button.
- For the pathogen TLF sensor we just need to press start for launching the measurement and we will have a result on the screen.
- Both options allow the “CLEAR SCREEN” and the “SAVE TO USB” buttons to clear the data already shown on the app and saving the data measured in a USB stick.

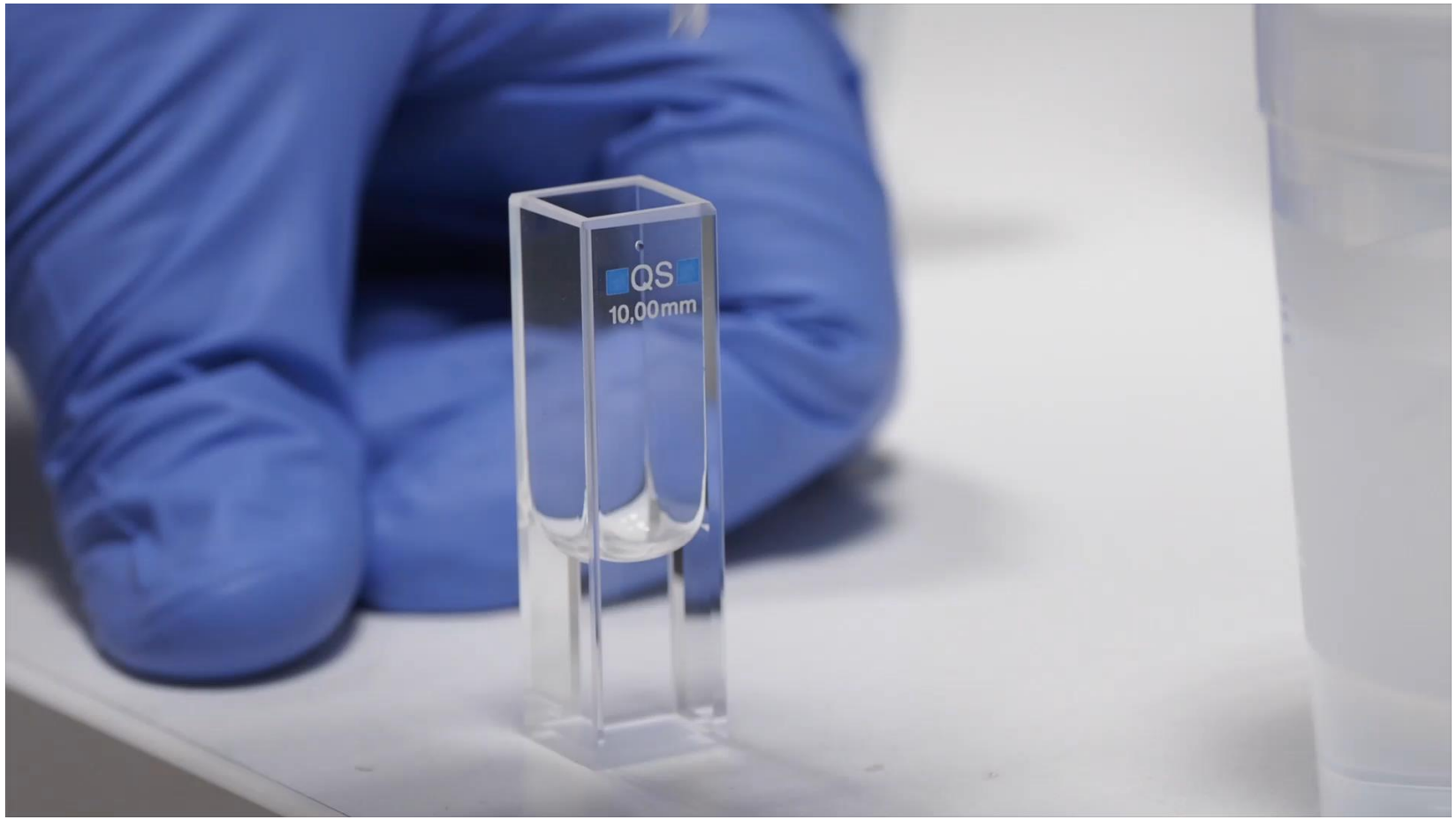


VFA sensor app display



Pathogen sensor app display

Sensors operation interface (VIDEO)



Example:
the PAVITR pilot project

PAVITR pilot in Aligarh (India)

Pathogen sensor for E.coli – Field valdiation

On field start- up

- Sensors arrived at Aligarh Muslim University (Field validation 1 year)
- The sensor was assembled, software updated and all the elements were checked on the field.
- Initial tests on field and adjustments in treated water in AMU.

PAVITR pilot in Aligarh (India)

Pathogen sensor for E.coli – Field valdiation

- The suitcase device was taken into the field for measuring the wastewater filtrated in the TTZ's Short Rotation Plantation (SRP) in AMU plant.
- The portable configuration allowed for live measurement on the sampling point.

Water sampling from SPR. AIMEN and TTZ

Short Rotation Plantation (SRP). Aligarh (India)

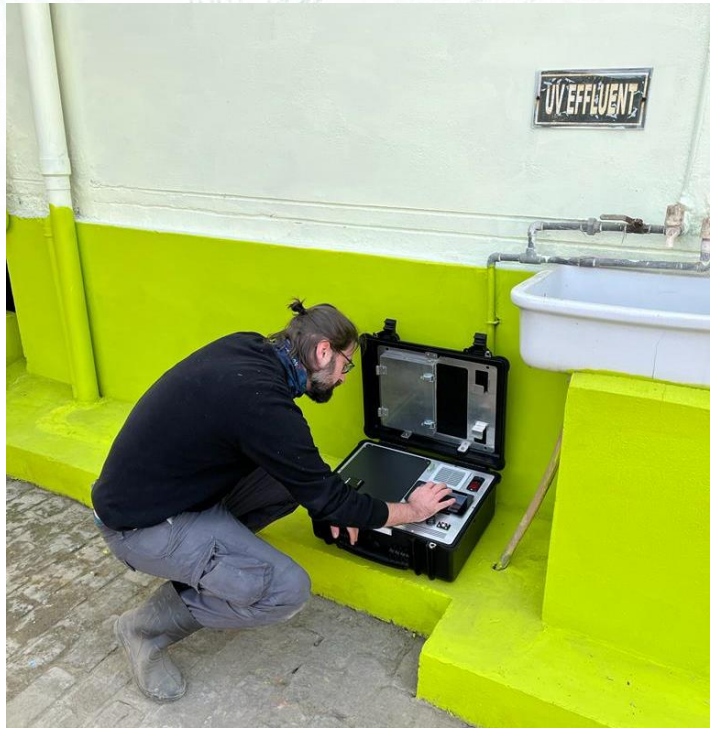
PAVITR pilot in Aligarh (India)

Pathogen sensor for E.coli – Field valdiation

Different water samples from willow (W), bamboo (B) and poplar (P) ground were measured using the sensor.
Sensor results is shown in cfu/100 ml (*cfu: colony forming units). Voltage signal from the photomultiplier was also aquired from from the data in order to recalibrate for AMU Plant wáter matrix.

Sample	Signal (V)			cfu/100 ml (Sensor)		
Blank	0,065	0,051	0,071	0	0	0
	0,062333333			0		
RG	0,374	0,365	0,385	92,06	88,29	94,99
	0,374666667			91,78		
BC	2,552	2,6	2,554	1012,3	1019,83	1016,9
	2,568666667			1016,343333		
BF	0,454	0,459	0,457	125,13	126,39	126,39
	0,456666667			125,97		
WC	0,513	0,506	0,499	149,83	146,9	143,97
	0,506			146,9		
WF	0,439	0,424	0,433	119,29	112,99	116,76
	0,432			116,3466667		
PC	0,753	0,792	0,754	249,8	266,22	250,31
	0,766333333			255,4433333		
PF*	4,635	-	-	1868,9		
	4,635			1868,9		
Raw WW**	0,139	0,24	0,448	0	36,37	123,04

Results from SRP filtrated wáter analysis



Sensor calibration in UV Effluent tap in Aligarh (India)

PAVITR pilot in Aligarh (India)

Pathogen sensor for E.coli – Field valdiation

Different water samples from willow (W), bamboo (B) and poplar (P) ground were measured using the sensor.

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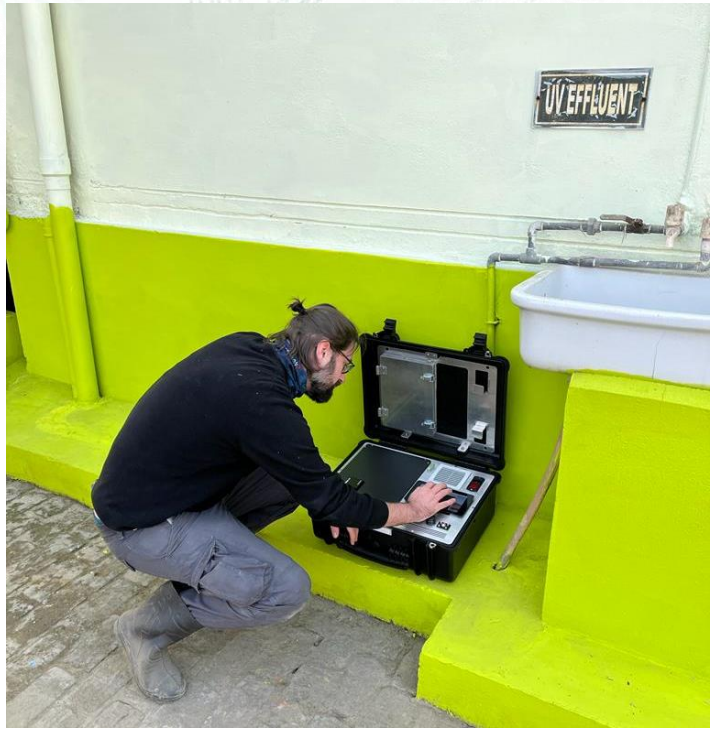
BF	0,454					
						143,97
					146,9	
				119,29	112,99	116,76
				116,3466667		
		0,792	0,754	249,8	266,22	250,31
	0,766333333			255,4433333		
PF*	4,635	-	-	1868,9		
	4,635			1868,9		
Raw WW**	0,139	0,24	0,448	0	36,37	123,04

Coliforms concentration data from the sensor needs to be crosschecked in a laboratory in order to re-calibrate the sensor for AMU water matrix.

Results from SRP filtrated wáter analysis



Sensor calibration in UV Effluent tap in Aligarh (India)

PAVITR pilot in Aligarh (India)

VFA sensor for Up-flow Anaerobic Sludge Blanket (UASB) – Field validation.

- Validation on the field of the VFA sensor sampling UASB effluent
- Estimated VFA concentration value from the sensor in order to monitor UASB
- Sensors operation training to AMU personnel

VFA sensor deployed in AMU plant Aligarh (India)

Sensors operation training to AMU personnel. Aligarh (India)

UASB reactor in PAVITR project. Aligarh (India)

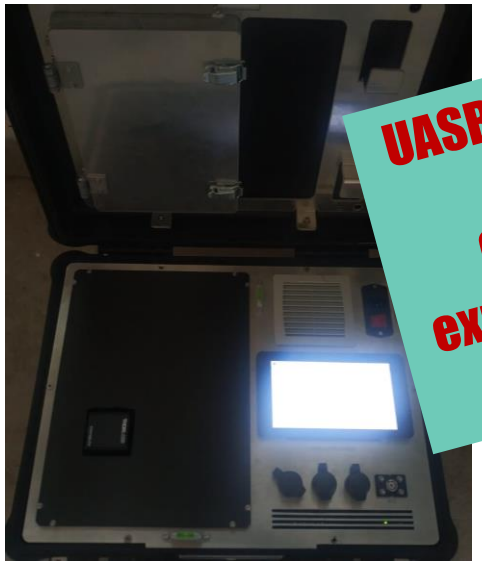
PAVITR pilot in Aligarh (India)

VFA sensor for Up-flow Anaerobic Sludge Blanket (UASB) – Field validation.

- Validation on the field of the VFA sensor sampling UASB effluent
- Estimated VFA concentration value from the sensor in order to monitor UASB
- Sensors operation training to AMU personnel

UASB reactor not operative yet: no measurement posible.

On January 2023 the sensor was tried in Aligarh but experimented electronic malfunction. Not operative until June 2023.



VFA sensor deployed in AMU plant Aligarh (India)

Sensors operation training to AMU personnel. Aligarh (India)

UASB reactor in PAVITR project. Aligarh (India)

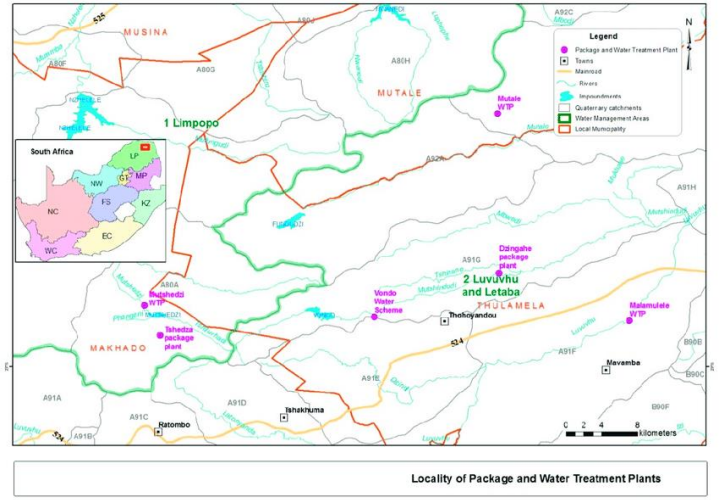
Homework

Exercise to design/implement the technology for a case study

Introduction to the case study

There is the need to deploy one of our sensors in an area were several water disinfection systems are scattered in different villages through a big region.

The aim is to ensure the water disinfection quality for all the people supplied by this network.



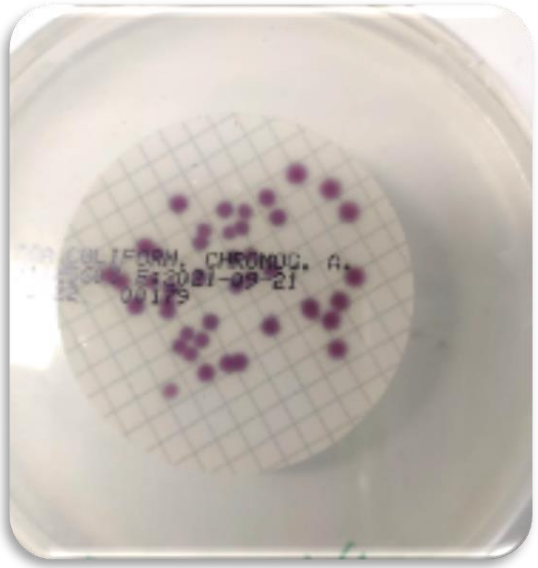
The Limpopo Non-Metropolitan Drinking Water Supplier Response to a Diagnostic Tool for Technical Compliance DO - 10.3390/ijerph14070810

AUTARCON wáter disinfection plant in Delhi (India)

Sustainable Water Plant – A step towards developing communities in Africa (shalina.com)

Key data for calculations

- E. Coli limit (cfu/100 ml) depending on the water use objective (drinking, crops irrigation, other uses, etc.)
- The level of water chlorination
- Power availability for charging

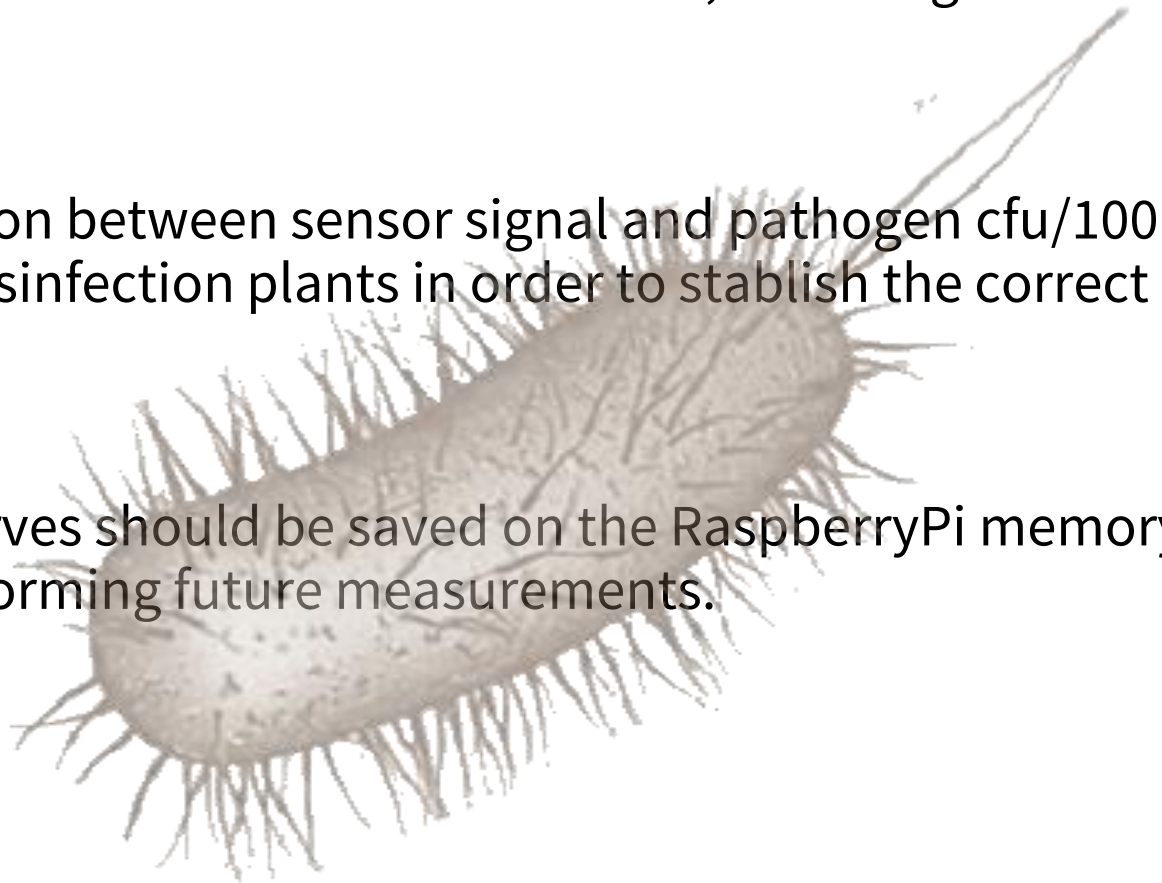


It is important to calibrate the sensor for each water disinfection plant, as the minerals, turbidity, chlorination level or other suspended components in water may change.

This calibration should saved in the sensor's RaspberryPi in order to estimate the cfu/100 ml coliforms concentration

Your homework is

- Plan the sensor validation for all different sites, including all the measurements you need.
- Find the correlation between sensor signal and pathogen cfu/100 ml for all different water disinfection plants in order to stablish the correct sensor calibration.
- All calibration curves should be saved on the RaspberryPi memory in order to be available for performing future measurements.



References

- Edberg, S.C., Rice, E.W, Karlin, R.J. and Allen, M.J., (2000): Escherichia coli: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology 2000, 88, 1068-1168. The Society for Applied Microbiology

Credits

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