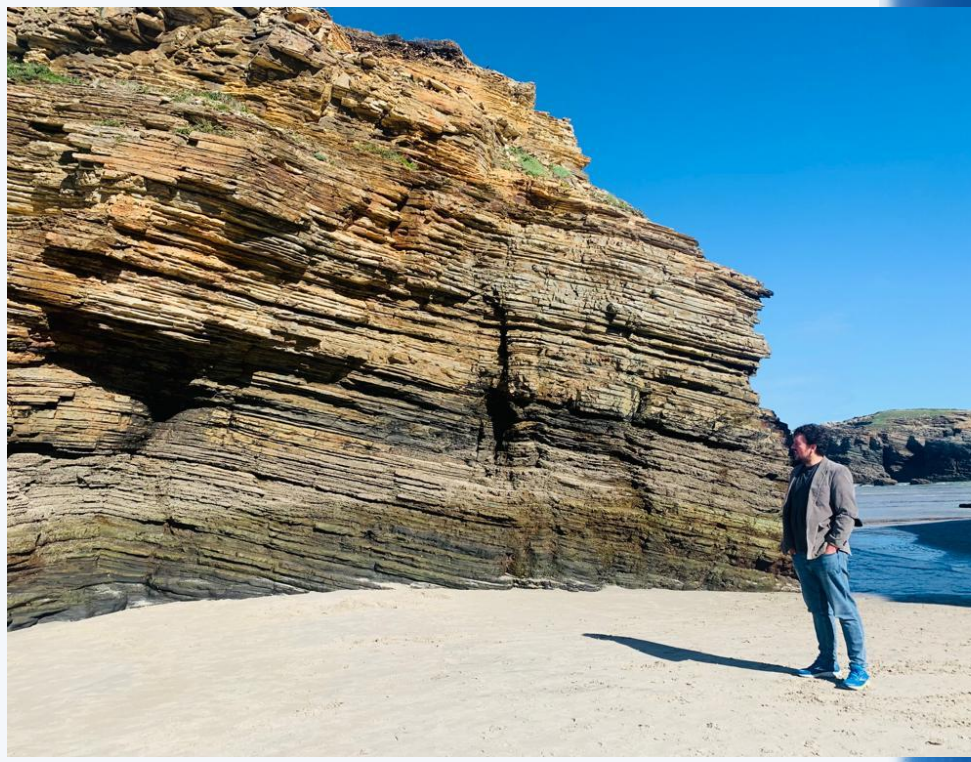
Real time monitoring of anaerobic fermentation by Raman and FTIR spectroscopy

Miguel Bouzas, Manuel de la Fuente, Juan Cubero-Cardoso, **Miguel Mauricio-Iglesias**

CRETUS, Department of Chemical Engineering
Universidade de Santiago de Compostela

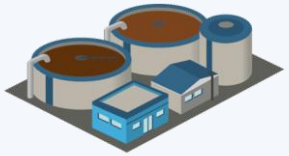


Praia das Catedrais
1h30 from Santiago de Compostela

Why anaerobic fermentation to recover carbon?

In anaerobic fermentation, the organic carbon is converted into volatile fatty acids

carbon rich residues

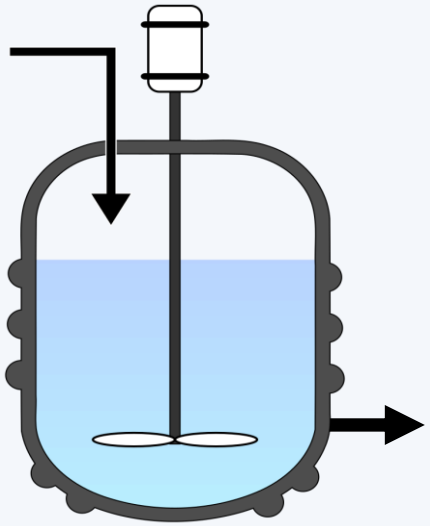


sludg

thin stillage

whey

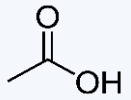
food processing wastewater



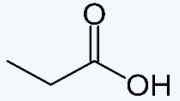
pH

SRT

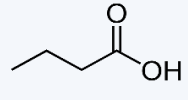
VFA & alcohols



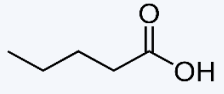
Acetate



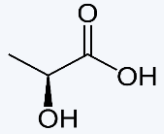
Propionate



Butyrate



Valerate



Lactate

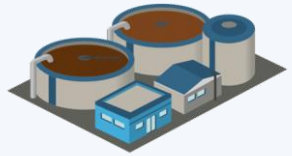
Ethanol

It is an open mixed-culture fermentation steered by pH, HRT and SRT, substrate composition, feeding mode, etc.

One of the main obstacles in utilizing VFA from anaerobic fermentation is that you do not get a product but a mixture

In anaerobic fermentation, the organic carbon is converted into volatile fatty acids

carbon rich residues



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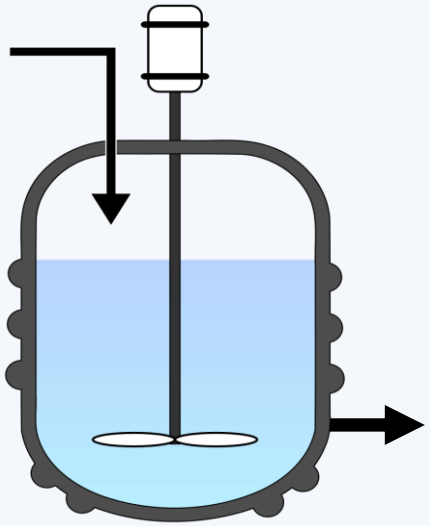
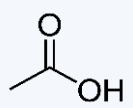
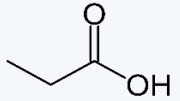


pH SRT

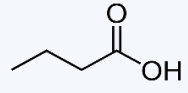
VFA & alcohols



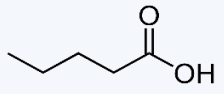
Acetate



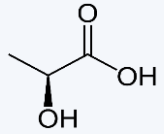
Propionate



Butyrate



Valerate



Lactate

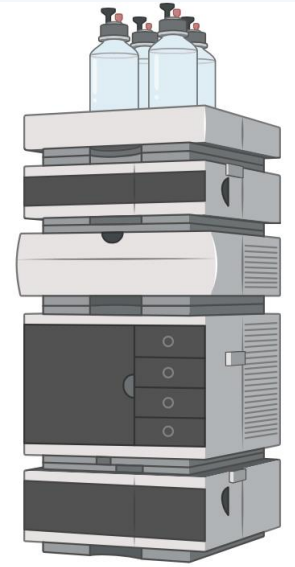
Ethanol

It is an open mixed-culture fermentation steered by pH, HRT and SRT, substrate composition, feeding mode, etc.

And monitoring these reactors, based on chromatography, misses fast dynamics and requires a lot of resources

Conventional method:

Gas & Liquid Chromatography



- Sample pretreatment
-Delay and mostly offline measurements

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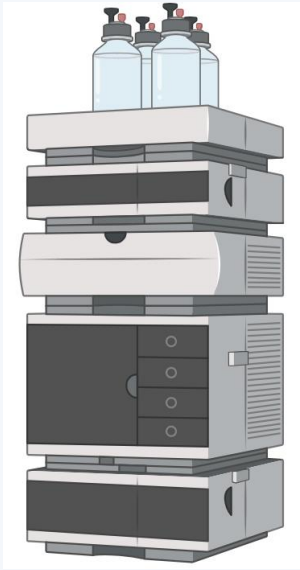
Gas & Liquid Chromatography



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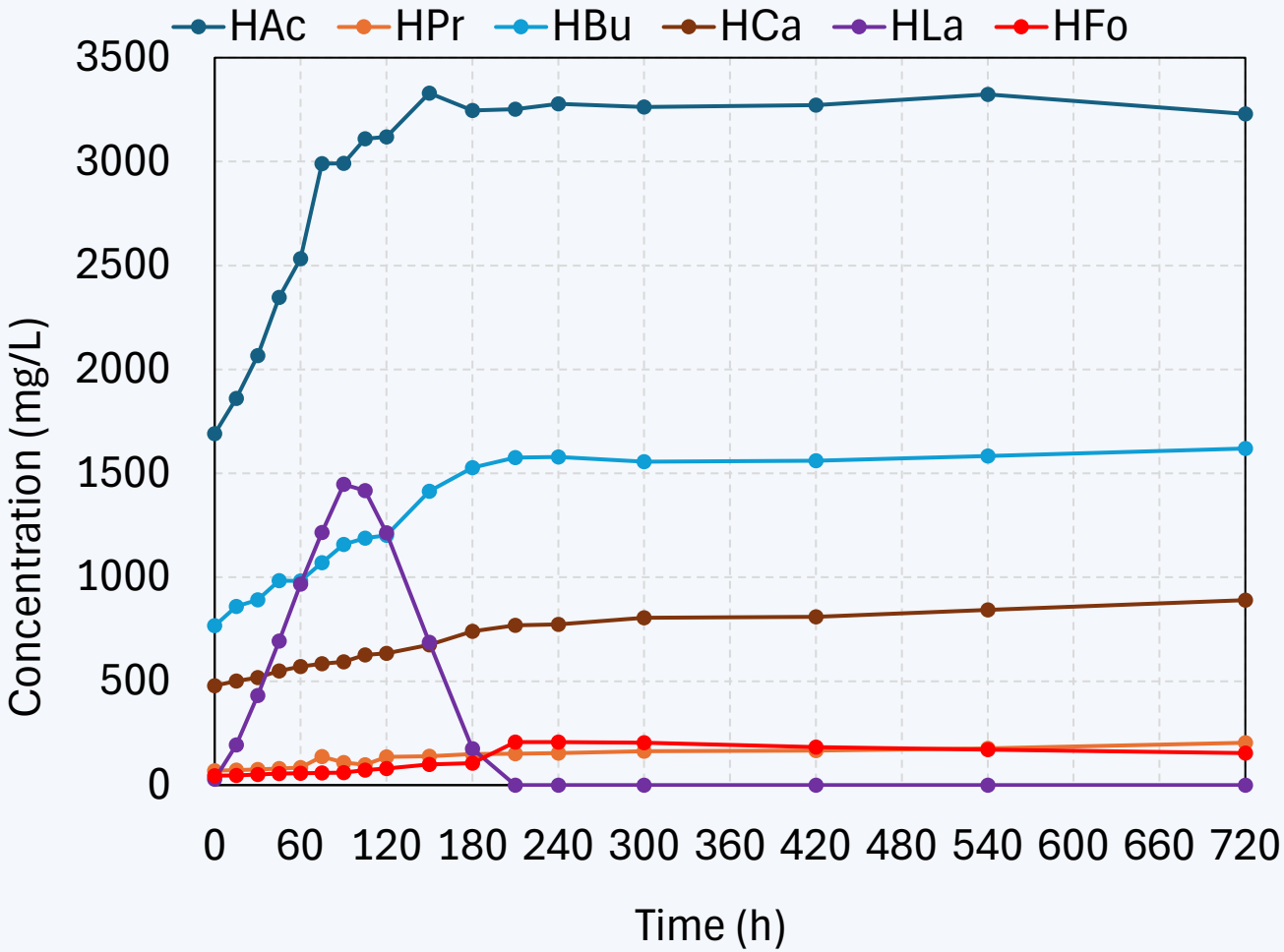

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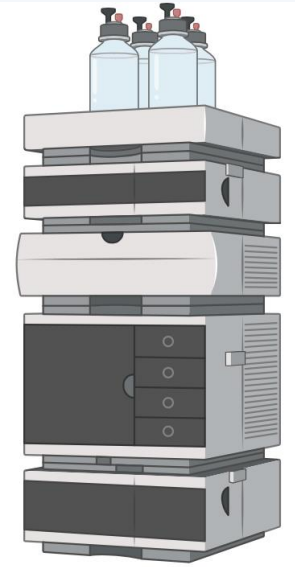
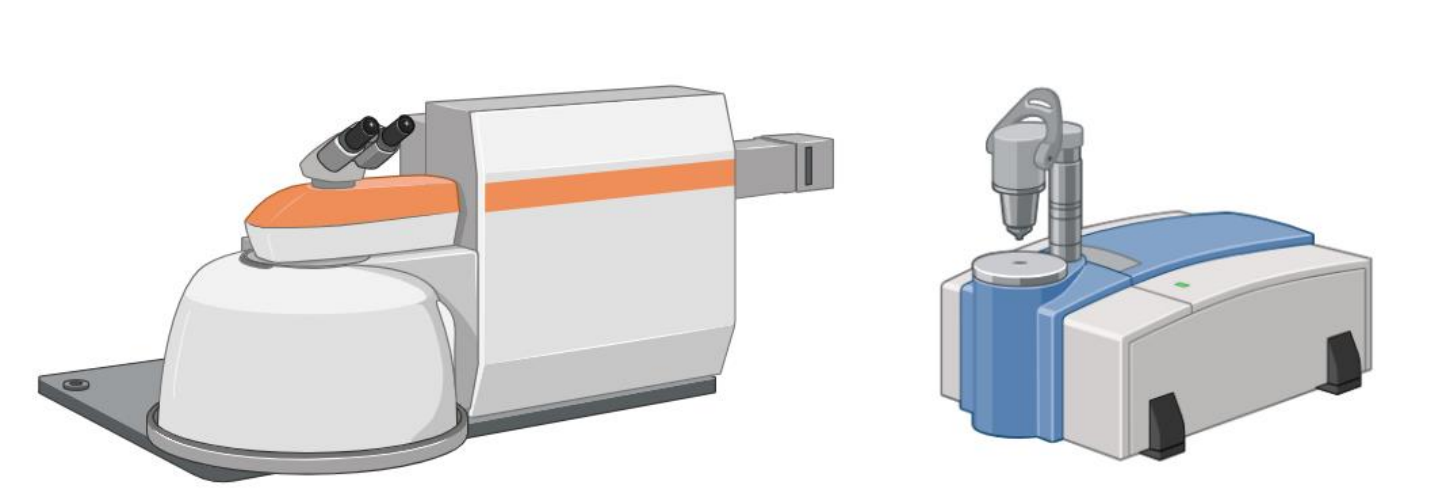


- Sample pretreatment
-Delay and mostly offline measurements

Alternative: **Infrared** (IR) and **Raman** spectroscopy, possibly combined with chemometric methods



-Possibility of enabling real time inline measurements
-Highly specifics and information rich
-<u>Lower sensitivity</u>

And monitoring these reactors, based on chromatography, misses fast dynamics and requires a lot of resources

Conventional method:

Alternative: **Infrared** (IR) and **Raman** spectroscopy, possibly

Gas

Are Raman and IR appropriate tools for anaerobic fermentation monitoring, given the concentration ranges, water interference?

Is Raman or IR better adapted to this application?

Can they be adapted to real-time monitoring?

ements

-Delay and mostly offline measurements

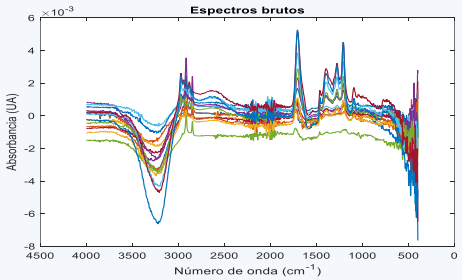
-Highly specifics and information rich

-<u>Lower sensitivity</u>

Methods: spectra acquisition, processing and data modelling

ATR-FTIR-MIR:
150 scans Res. 4 cm^{-1}

Raman 1064 nm:
3/20 exp., 15 s., 100% laser

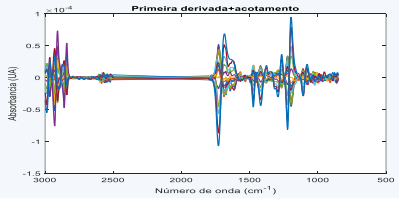


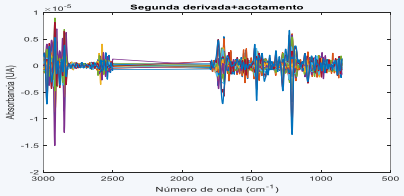
Centering

Smoothing – Savitzky-Golay

1st derivative

2nd derivative





Projection on Latent Structures (PLS) model

$$X = TP + E$$

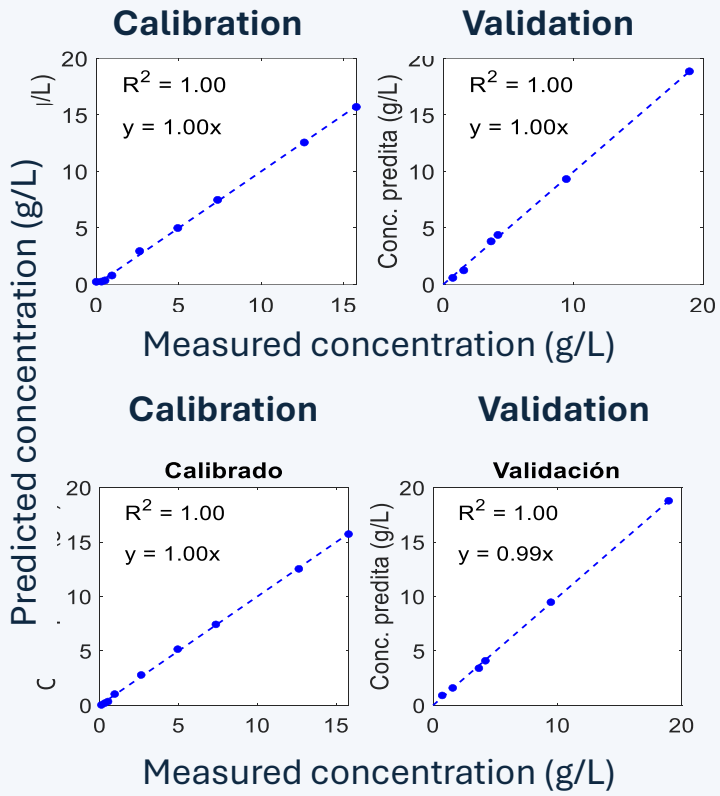
$$Y = ZQ + F$$

$$Z = \beta \cdot T$$

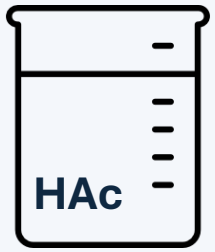
Validation (1/3 of data)

Acetate, propionate and butyrate can be measured by IR and Raman

First we checked whether the each of the analytes could be quantified separately



Acetic acid by IR

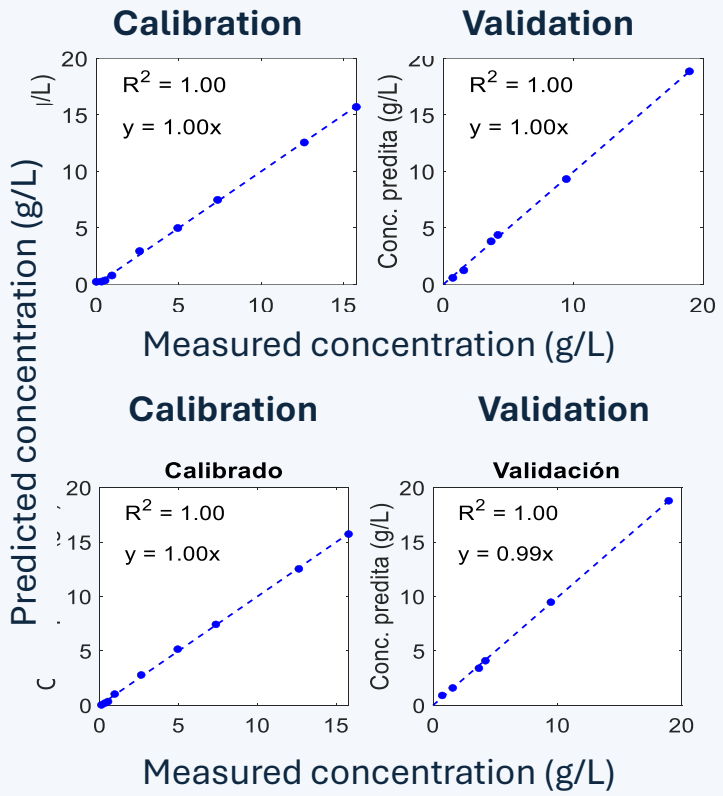


Acetic acid by Raman

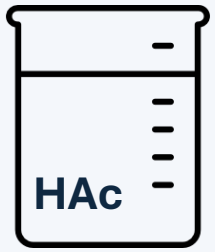
	m	R^2
HPro (IR/Raman)	1,00/0,96	1,00/0,99
HBut (IR/Raman)	0,99/1,02	1,00/0,99

Acetate, propionate and butyrate can be measured by IR and Raman

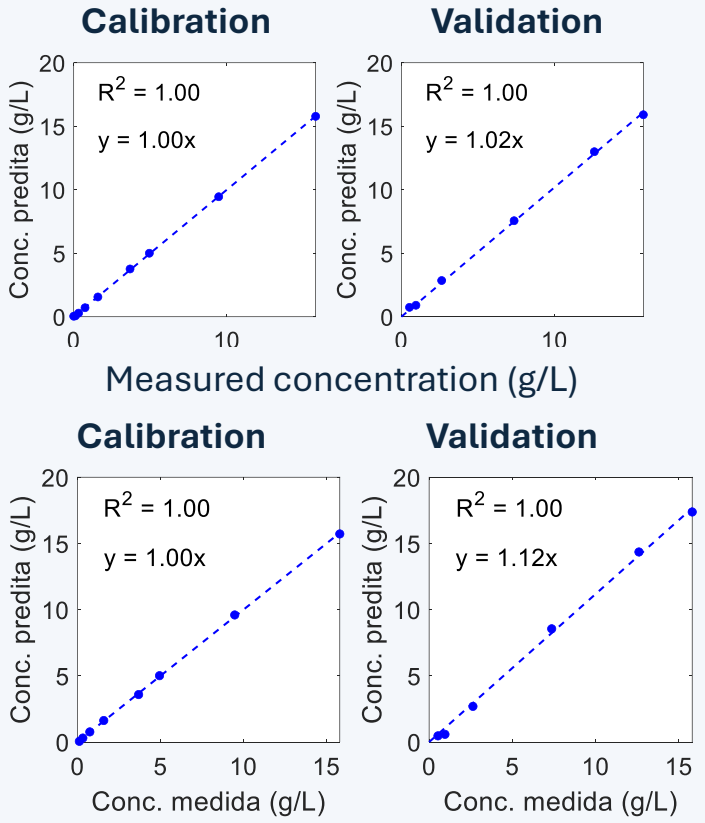
Also in aqueous mixtures of acetic, propionic and butyric acid



Acetic acid by IR



Acetic acid by Raman

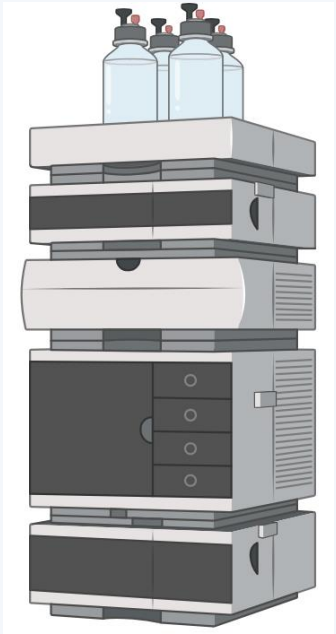


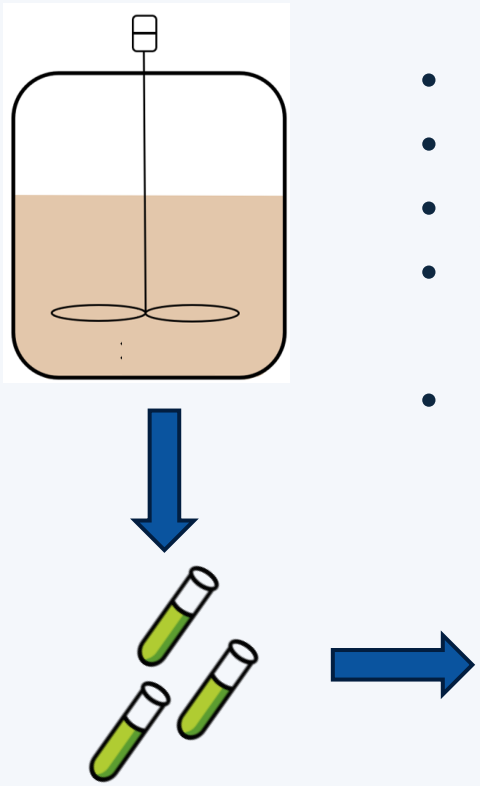
	m	R^2
HPro (IR/Raman)	1,00/0,96	1,00/0,99
HBut (IR/Raman)	0,99/1,02	1,00/0,99

	m	R^2
HPro (IR/Raman)	1,02/1,12	1,00
HBut (IR/Raman)	1,00/1,03	1,00

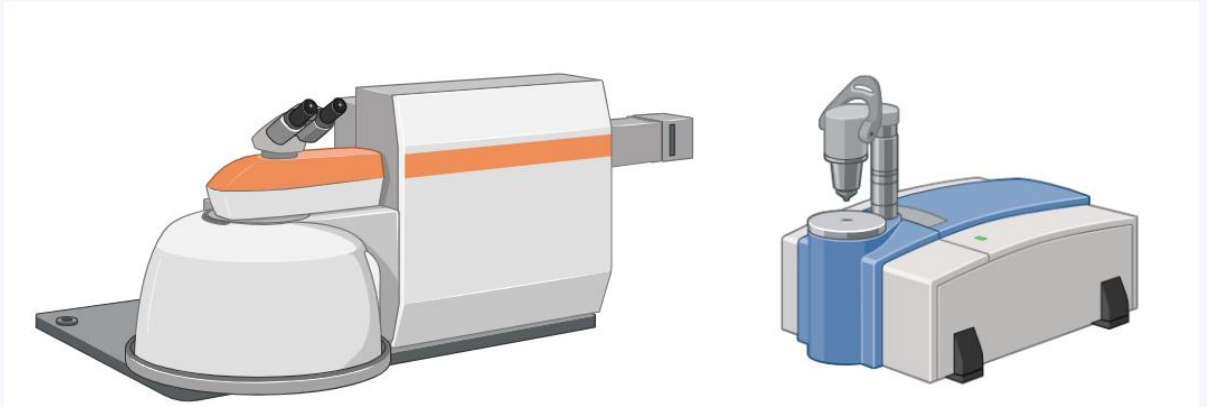
Now, let's monitor a fermentation using xylose as a substrate

Batch anaerobic fermentation tests x 3

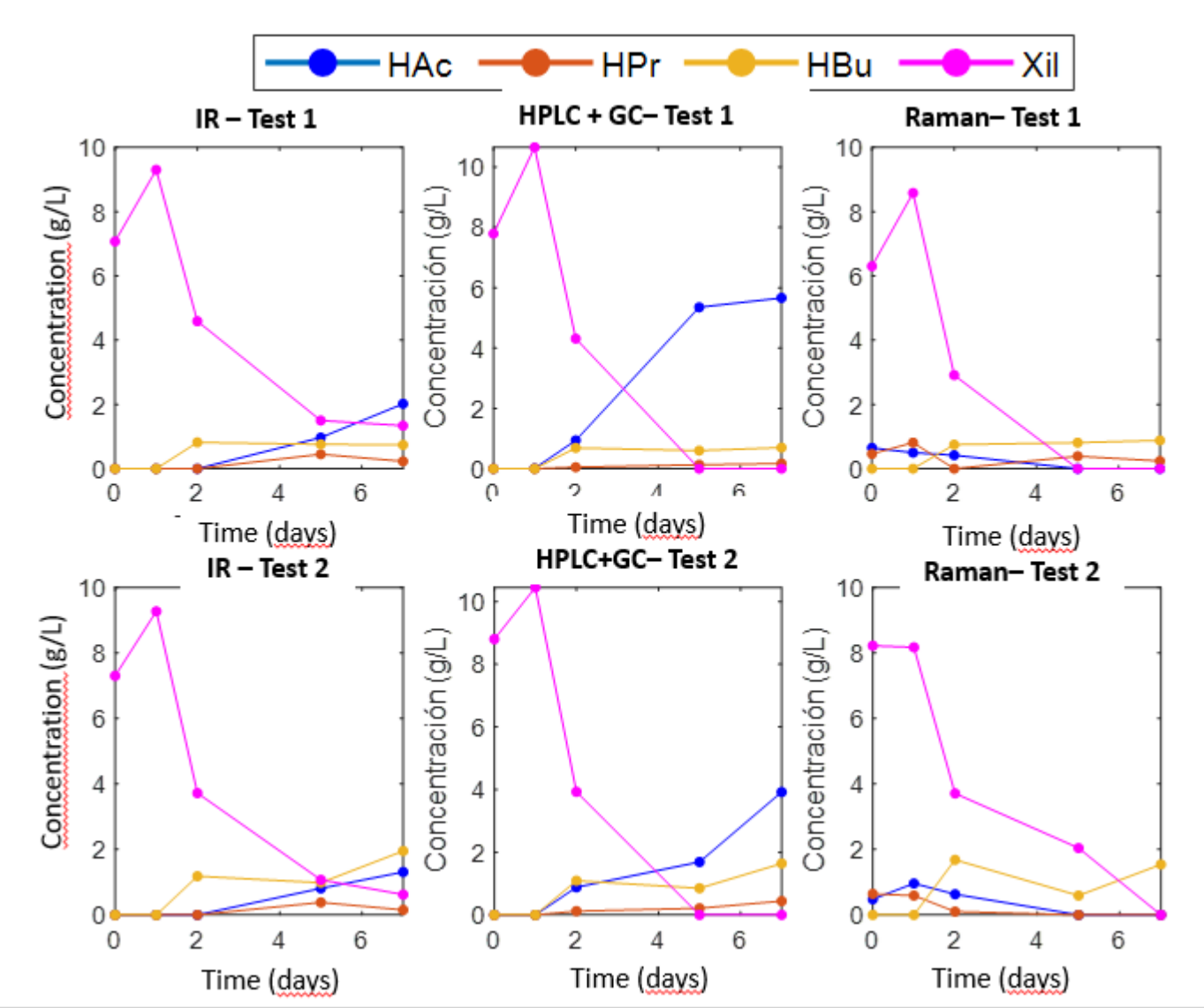




- T=37ºC – bicarbonate buffer + manual correction
- pH≈7
- Xylose 10 g/L
- Sludge from anaerobic fermentation as inoculum – BES for methanisation inhibition
- SIR= 2,00 g SV X/ g SV subs



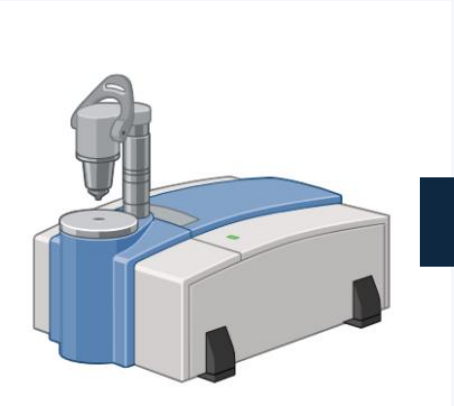
But when monitoring a fermentation the performance is not good enough

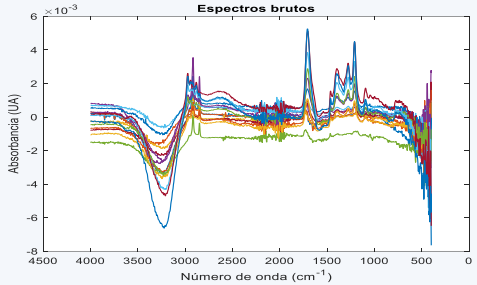


- Xylose predictions are largely correct
- Butyric acid is the only one with a low RMSE
- Propionic acid appears at too low concentrations to evaluate the PLS model (<0,45 g/L)
- Overall, IR performs better than Raman

	RMSE (g/L)
HAc (IR/Raman)	1.85/2.70
HPr (IR/Raman)	0.15/0.48
HBu (IR/Raman)	0.16/0.30

Integrating IR measurements with kinetic model information as a means of increasing the prediction accuracy

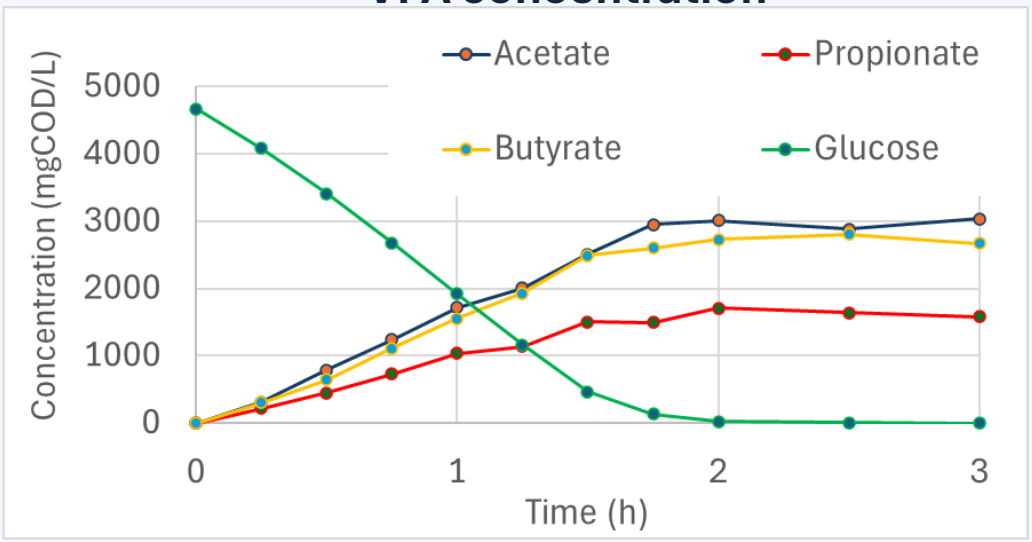




PLS model

HAc, HPro, HBut… substrate

VFA concentration



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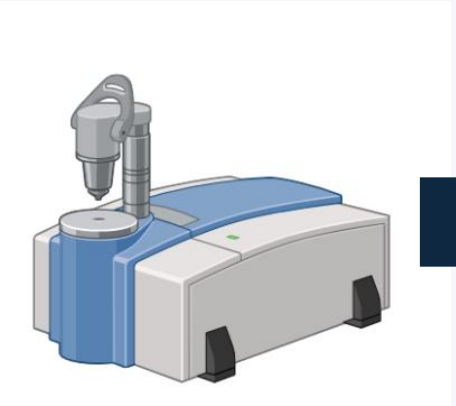

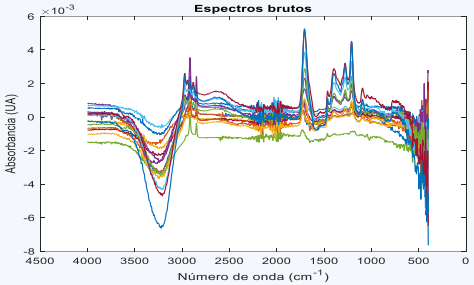

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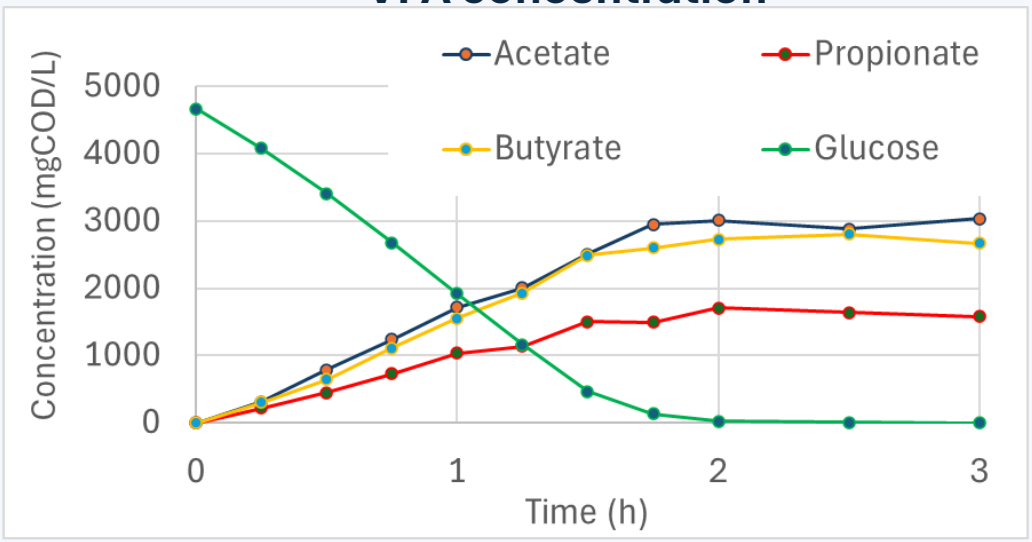
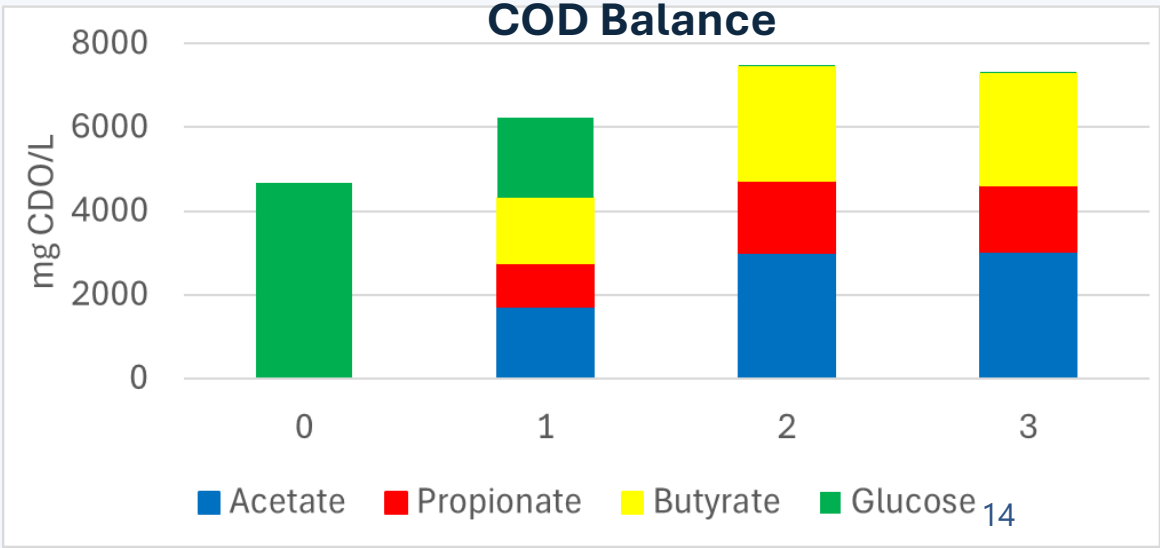
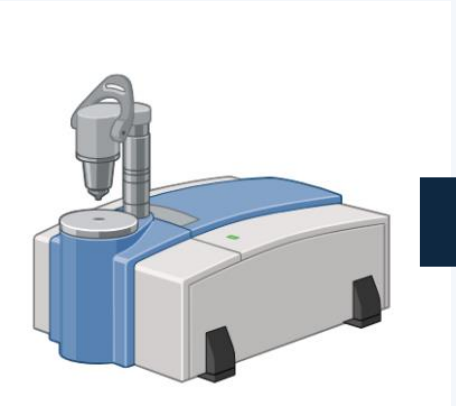
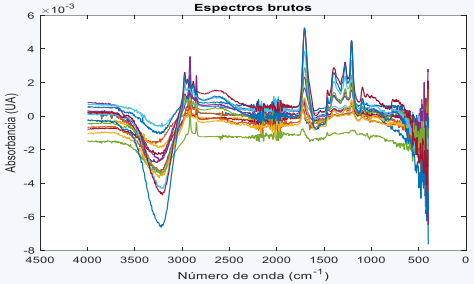


COD Balance



Integrating IR measurements with kinetic model information as a means of increasing the prediction accuracy





Fermentation mathematical model

Kinetic model predictions

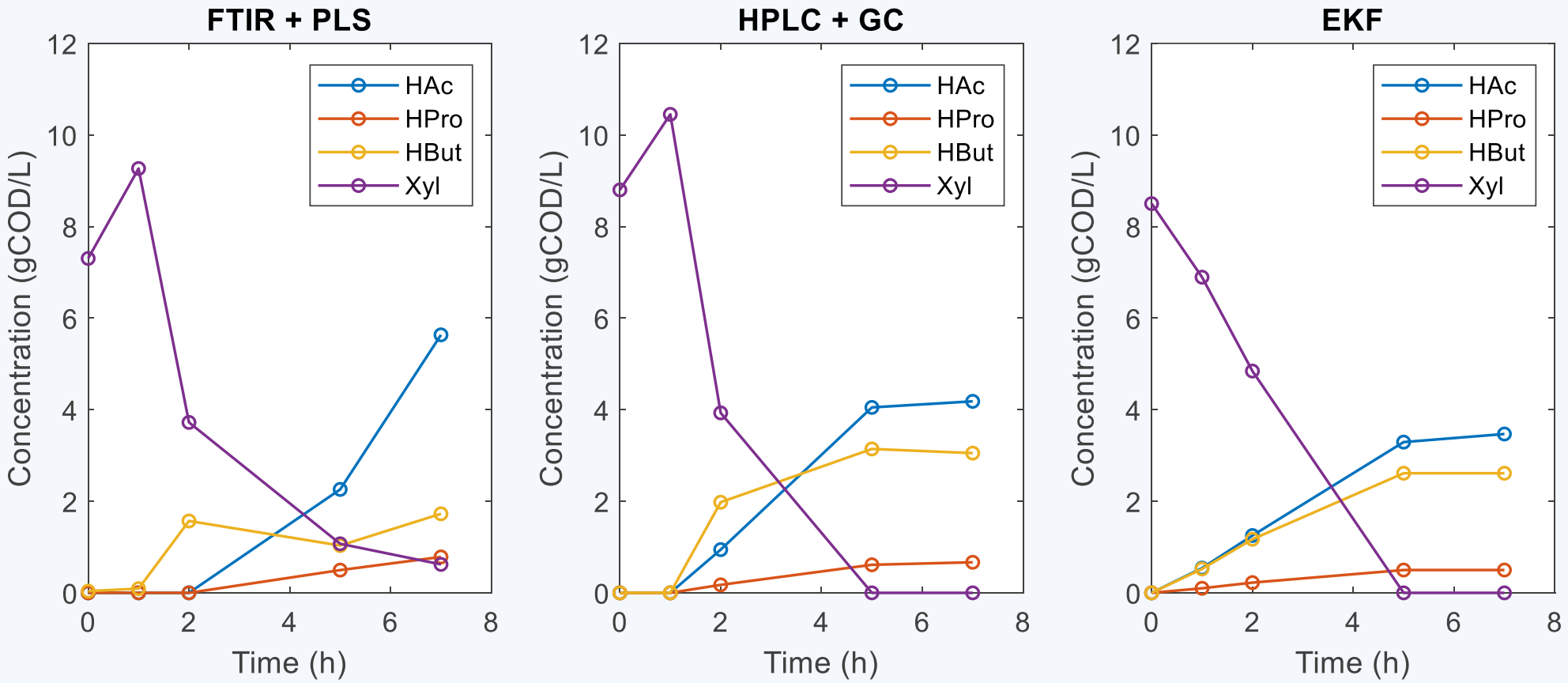
PLS model

Extended Kalman Filter

Measurements

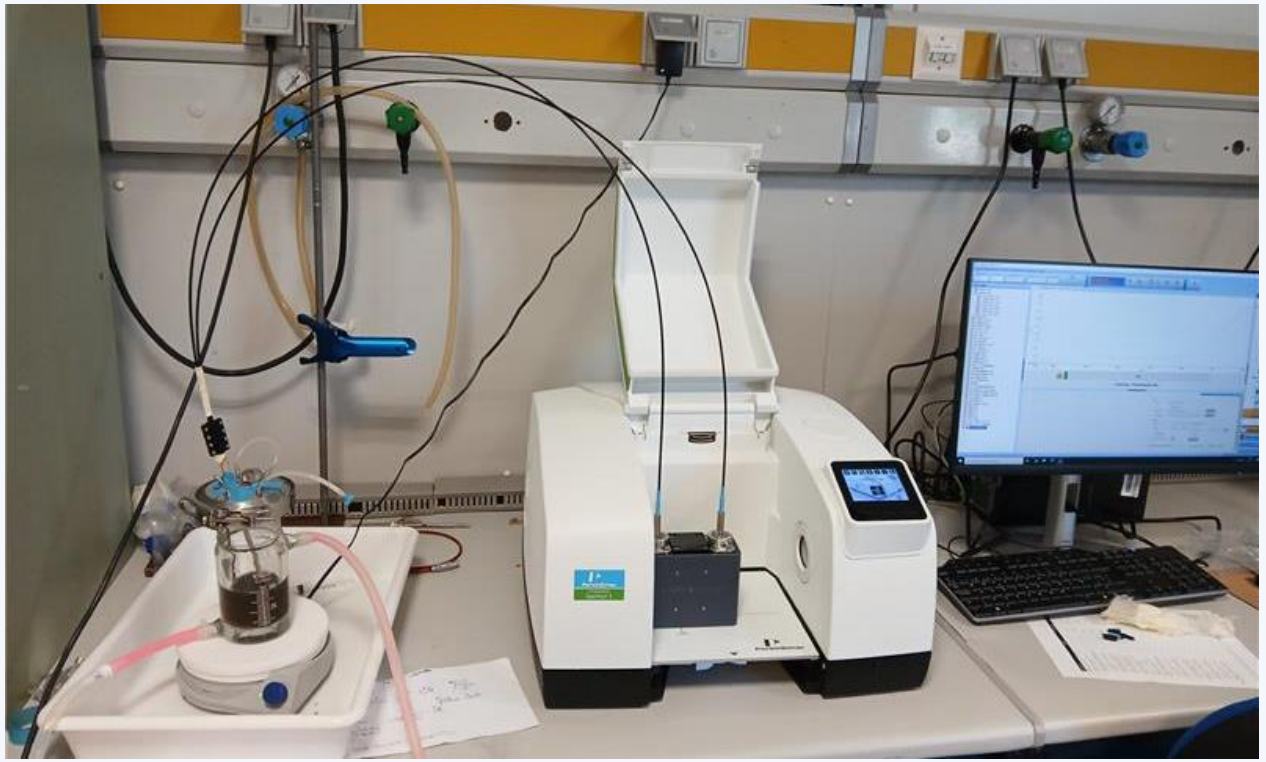
HAc, HPro, HBut… substrate

We use the Extended Kalman Filter for integration



	RMSE (g/L)
HAc (FTIR/EKF)	1.24 / 0.29
HPr (FTIR/EKF)	0.02 / 0.01
HBu (FTIR/EKF)	1.28 / 0.28

Can we move to real-time monitoring?



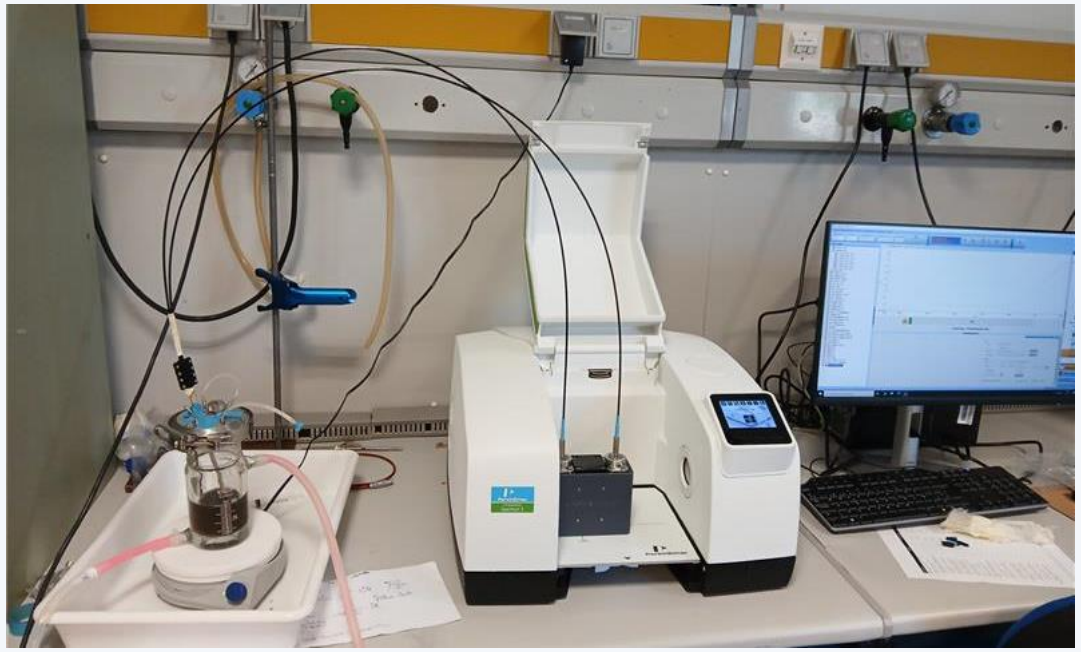
ATR diamond tip fiber probe
Spectral range 600-1900 cm^{-1}

We added an ATR diamond tip fiber probe. This probe limited the spectral range to 600-1900 cm^{-1}, still keeping the fingerprinting range but with a significant signal loss

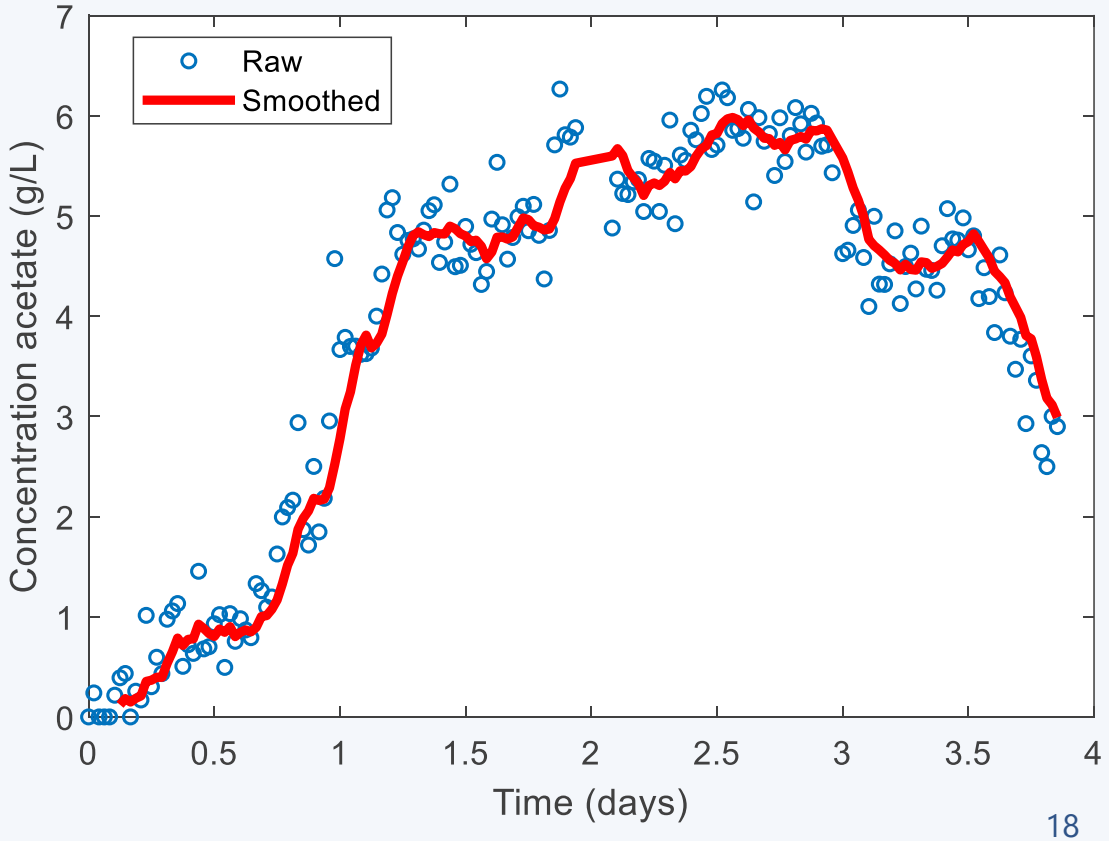
Batch anaerobic fermentation tests x 3

- T=37ºC – bicarbonate buffer + manual correction
- pH≈7
- Xylose 10 g/L
- Sludge from anaerobic fermentation as inoculum – BES for methanisation inhibition

Can we move to real-time monitoring?



We tried a fermentation and were able to follow acetate but there were many interferences due to failing pH control and unexpected methanisation



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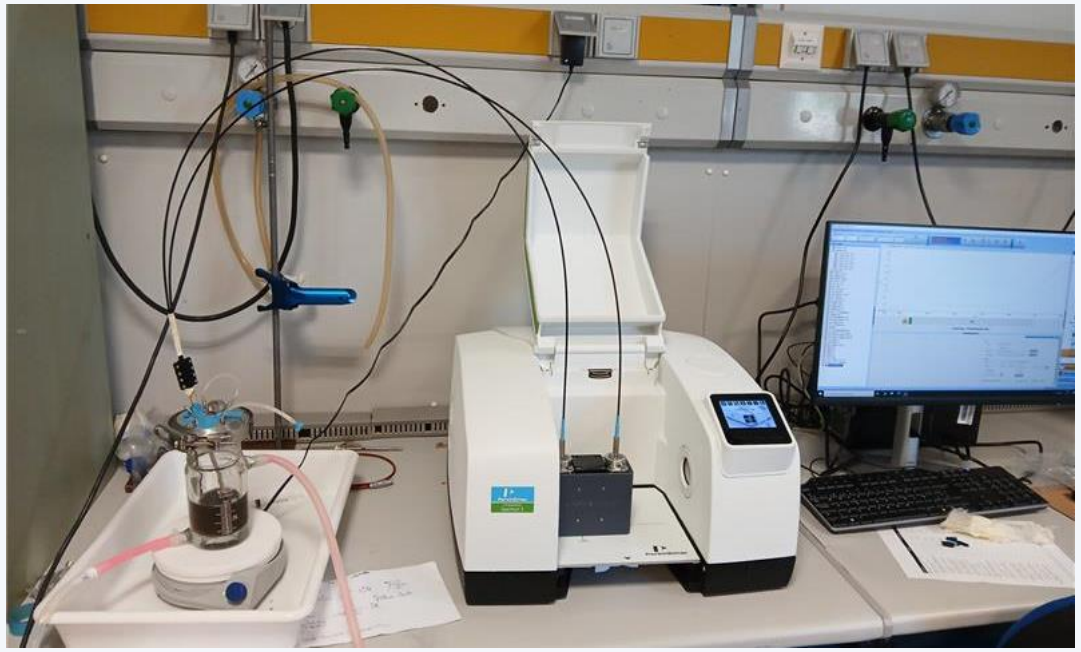

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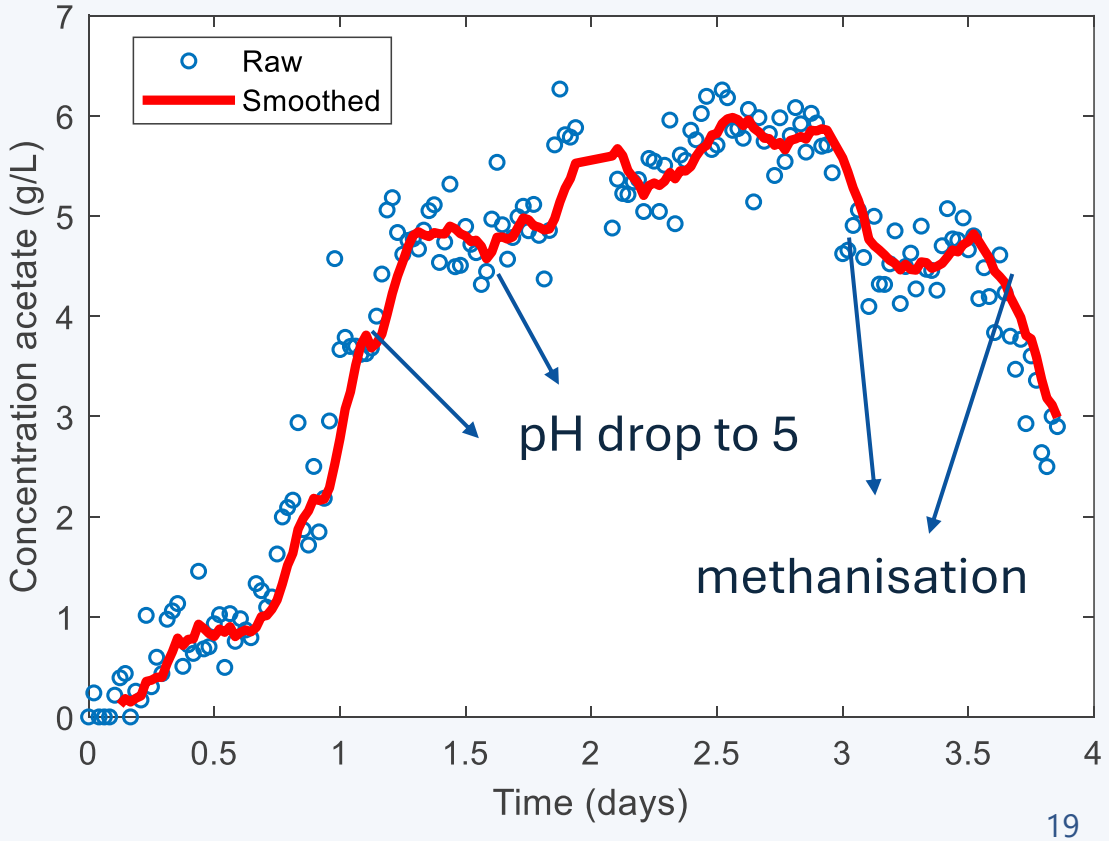

Take home messages and upcoming activities

1) Both IR and Raman were used to detect and quantify VFA (acetic, propionic and butyric acids) in fermentation. IR performed slightly better than Raman but both techniques could be appropriate

2) Integration with a model + nonlinear observer such as the extended Kalman Filter is an appropriate way to improve the prediction accuracy

3) Real-time monitoring using an ATR fiber probe is in principle possible but limited by the severe loss of signal caused by the fiber.

Want to know more? Come to Santiago de Compostela for the Carboxylates Symposium

Symposium

CHALLENGES OF THE BIOLOGICAL CONVERSION OF RESIDUES INTO CARBOXYLATES

Santiago de Compostela, 10th July 2025

Confirmed keynote speakers

Piotr Oleśkowicz-Popiel
Poznan University of Technology (Poland)

Maurice Oltheten
ChainCraft (The Netherlands)

Marta Carballa
Univ. Santiago de Compostela (Spain)

Sebastia Puig
University of Girona (Spain)

Information and registration (before June 30th):

miguel.mauricio@usc.es or **carboxylates@usc.es**

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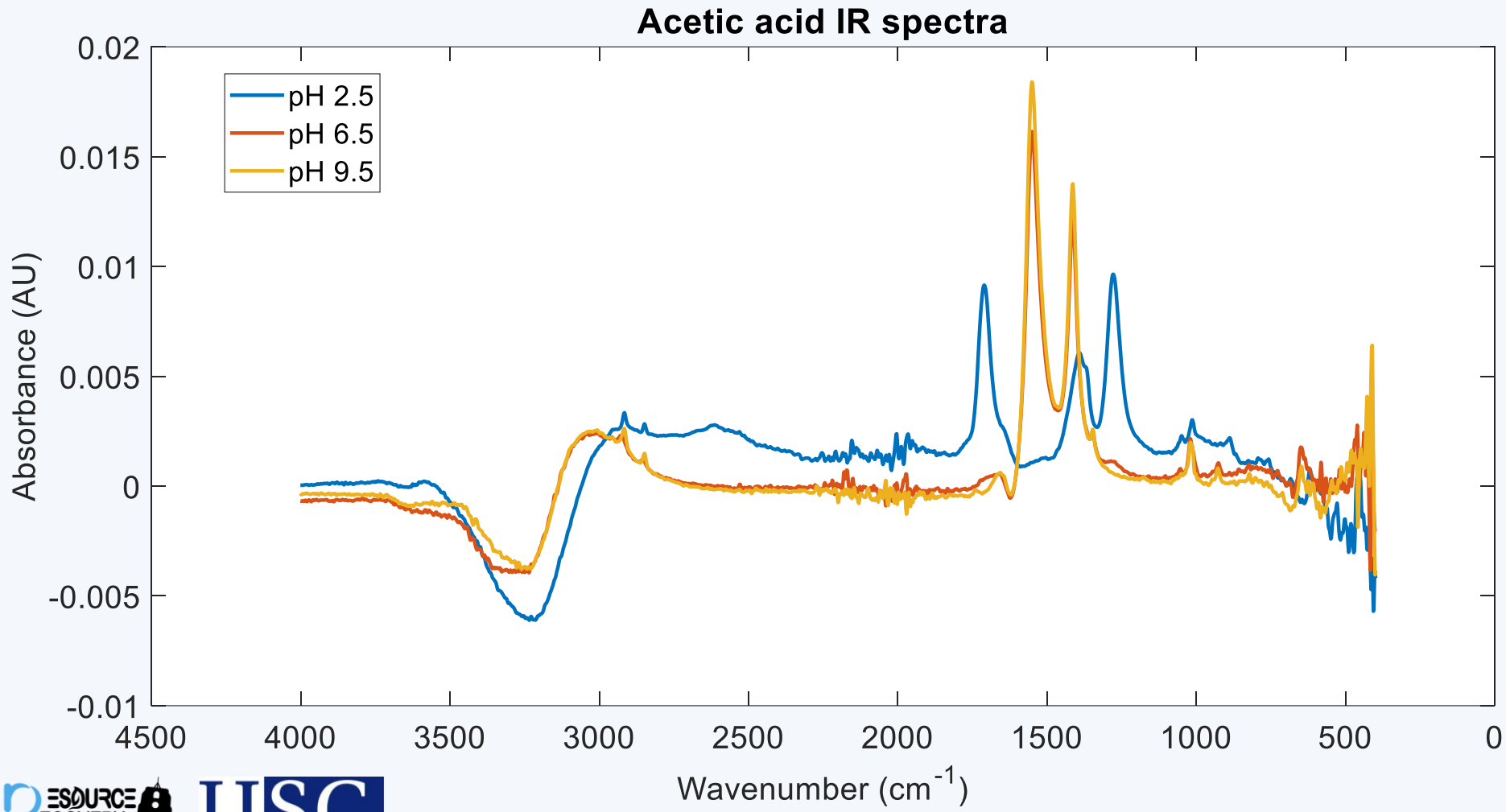
CRETUS, Department of Chemical Engineering
Universidade de Santiago de Compostela

Louro Beach
1h from Santiago de Compostela

Medium pH plays an important role here



- The peak at 1712 cm^{-1} (C=O stretching vibration of the carboxylic acid group) shifts to 1550 cm^{-1} at higher pH values.
- The peak at 1280 cm^{-1}, (C-O stretching vibration in the carboxylic acid group) shifts to 1415 cm^{-1}