Comparison of vibrational spectroscopy methods for real-time monitoring of anaerobic fermentation

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

CRETUS, Department of Chemical Engineering
Universidade de Santiago de Compostela

Why anaerobic fermentation?

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

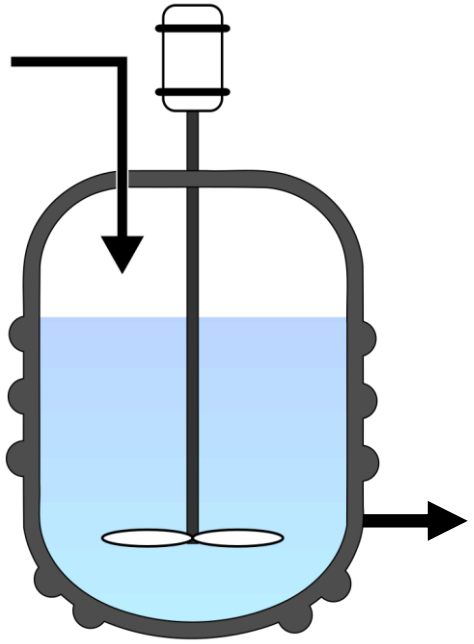
carbon rich residues

sludge

thin stillage

whey

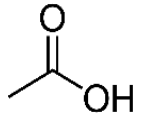
food processing wastewater



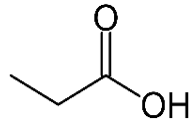
pH

SRT

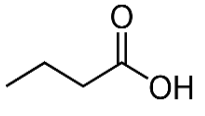
VFA & alcohols



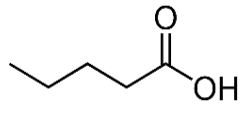
Acetate



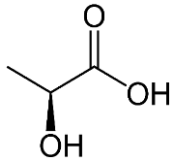
Propionate



Butyrate



Valerate

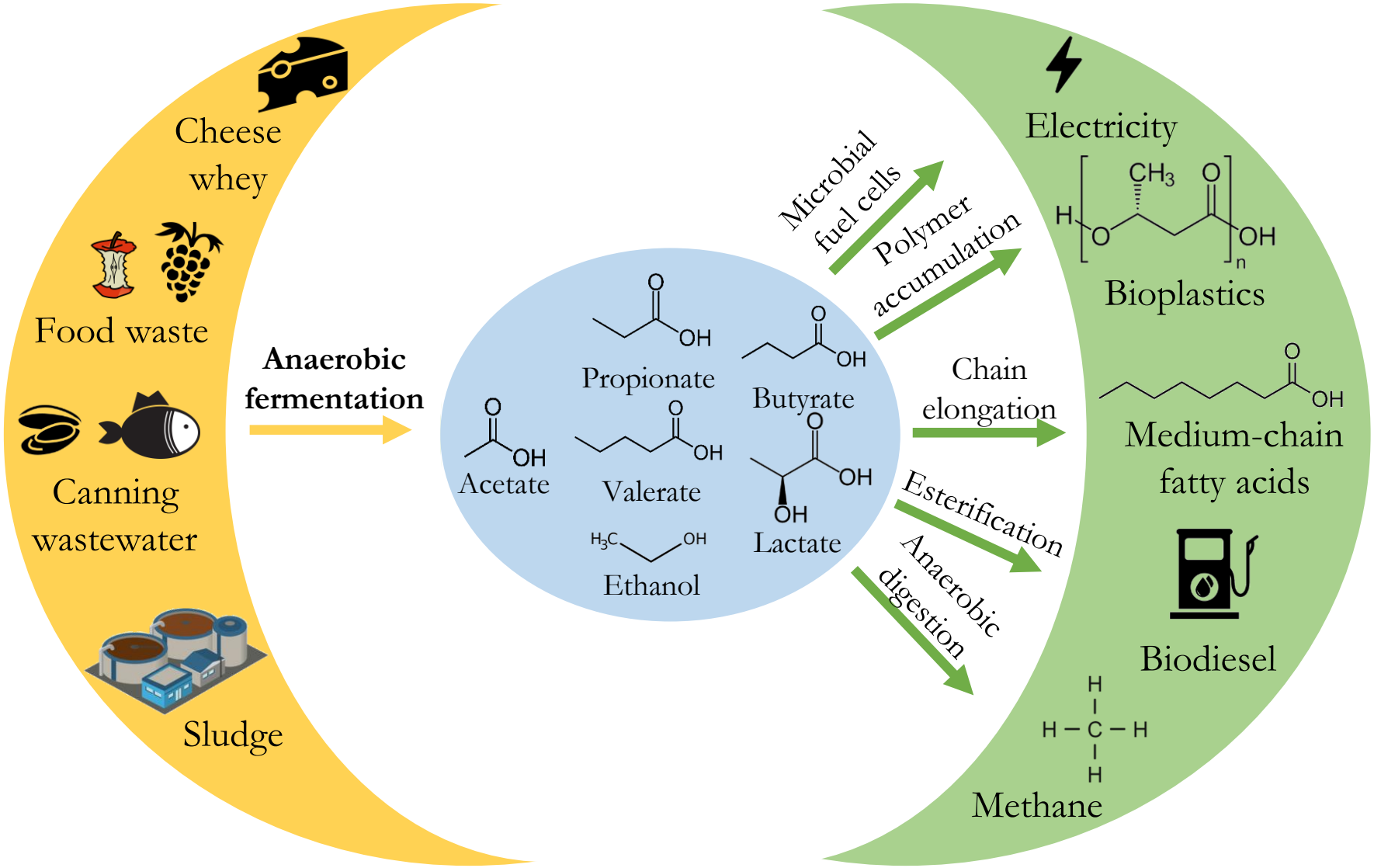


Lactate

Ethanol

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

Volatile fatty acids are platform chemicals → they can be further processed to produce many other chemicals and resources



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

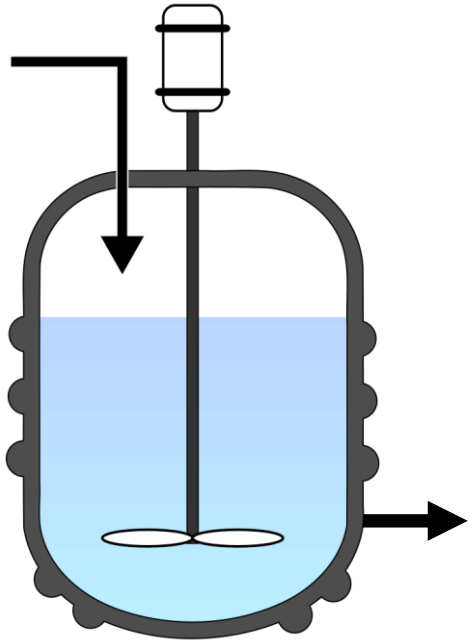
carbon rich residues

sludge

thin stillage

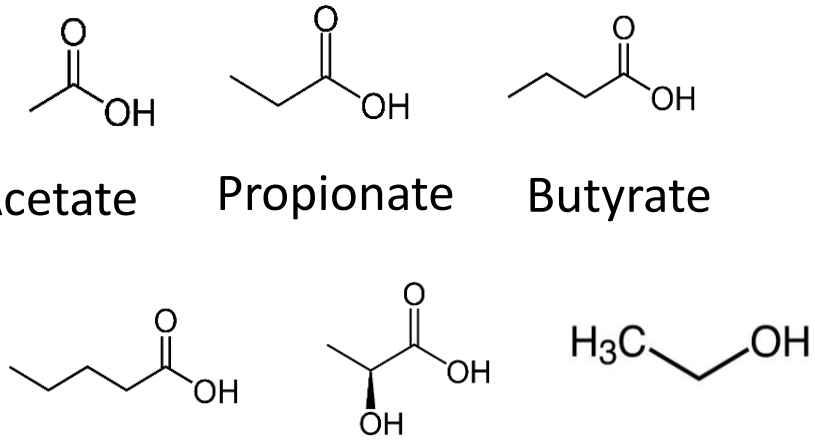
whey

food processing wastewater



pH SRT

VFA & alcohols



Acetate Propionate Butyrate

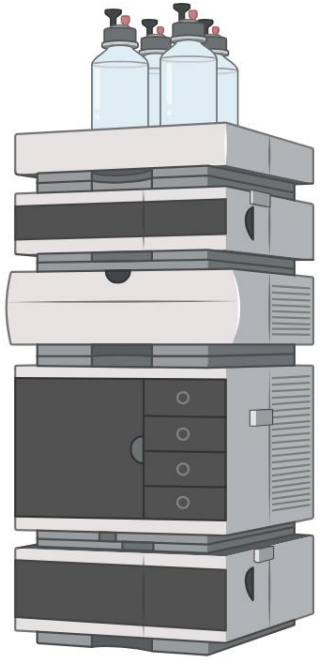
Valerate Lactate Ethanol

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

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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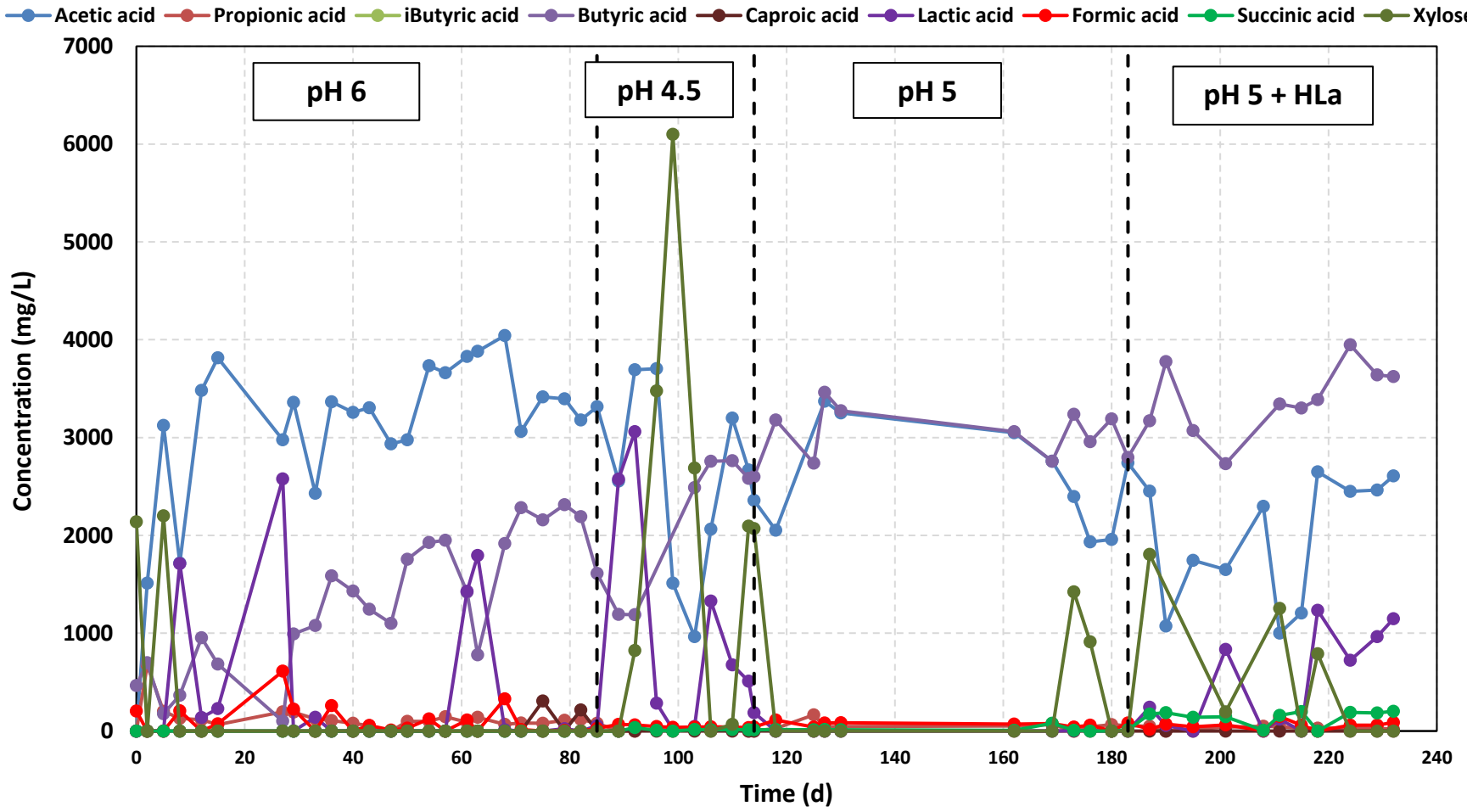

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Vibrational spectroscopy can be a tool for VFA quantification

Conventional method:
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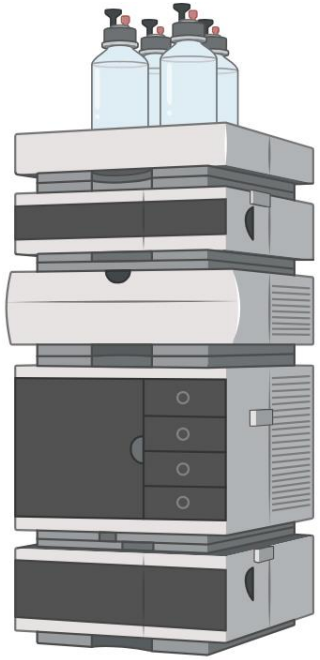
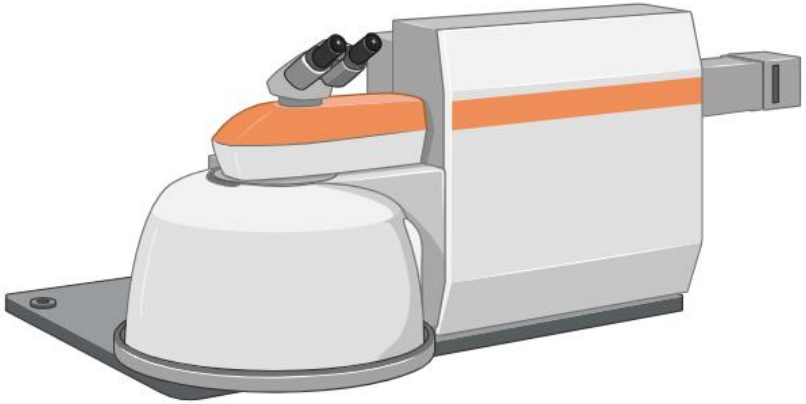
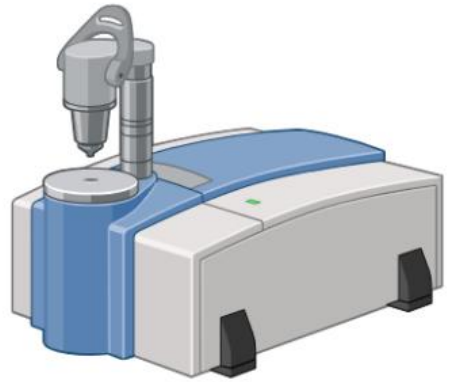


- Sample pretreatment
-Delay and mostly offline measurements

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>

Vibrational spectroscopy can be a tool for VFA quantification

Conventional method:
Gas & Liquid Chromatography

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

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?

- Sample pretreatment
-Delay and mostly offline measurements

-Possibility of enabling real time inline 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

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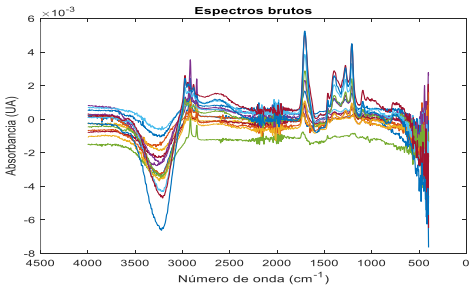
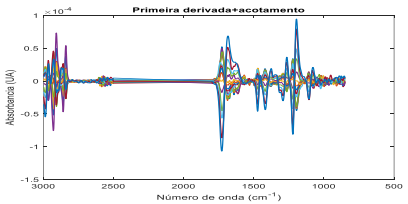


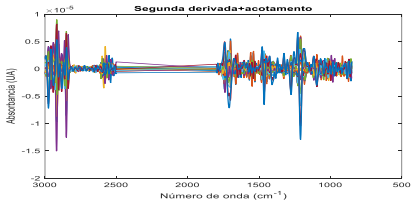
Centering

Filtering – Savitzky-Golay

1st derivative

2nd derivative





Projection on Latent Structures (PLS) model

$$X = TP + E$$

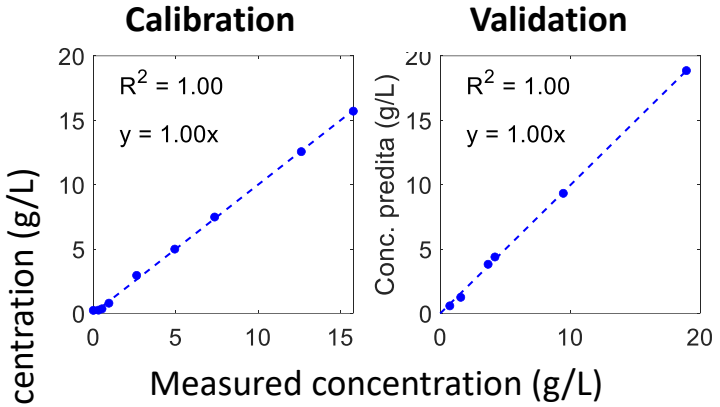
$$Y = ZQ + F$$

$$Z = \beta \cdot T$$

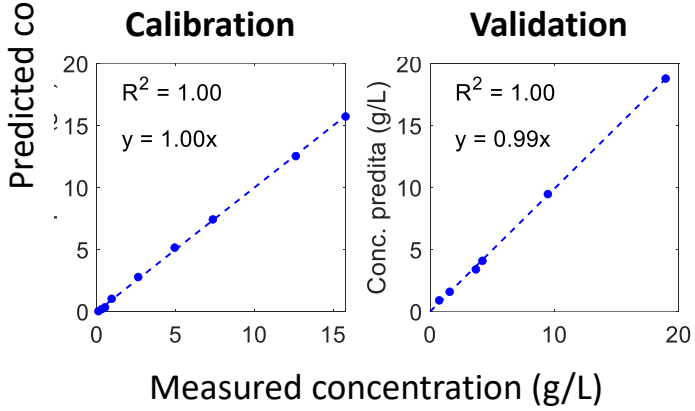
Validation (1/3 of data)

Acetate, propionate and butyrate can be quantified in water solutions

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



Acetic acid by IR

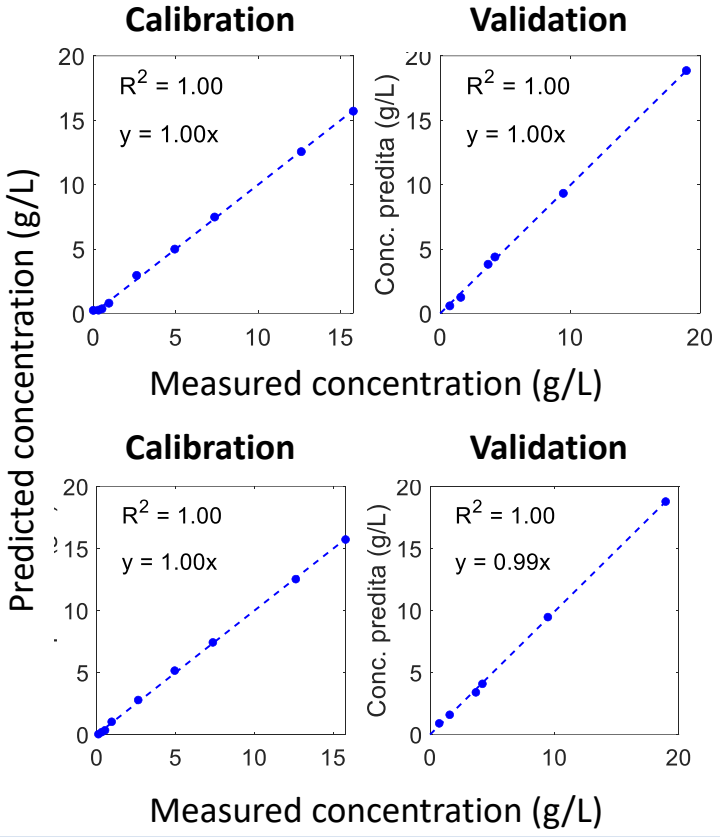


Acetic acid by Raman

	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

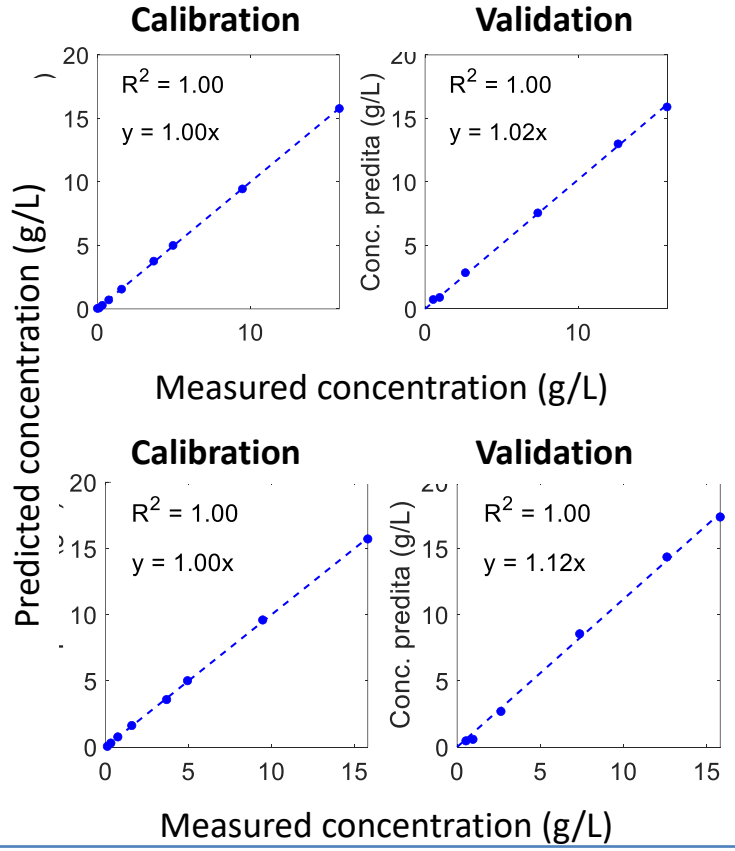
Acetate, propionate and butyrate can be quantified in water solutions

Also in aqueous mixtures of acetic, propionic and butyric acid



Acetic acid by IR

Acetic acid by Raman

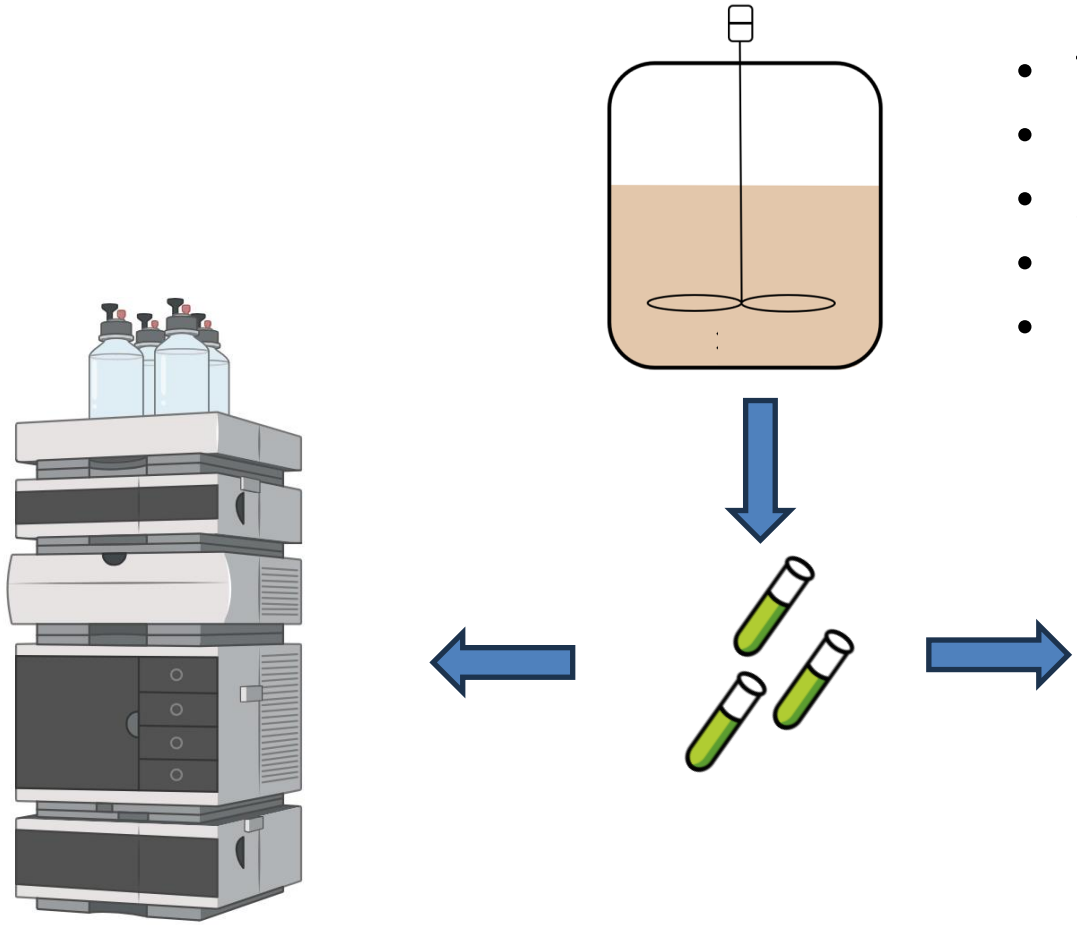


	m	R^2
HPro (IR/Raman)	1,00/0,96	1,00/0,99
HBut (IR/Raman)	0,99/1,02	1,00/0,99

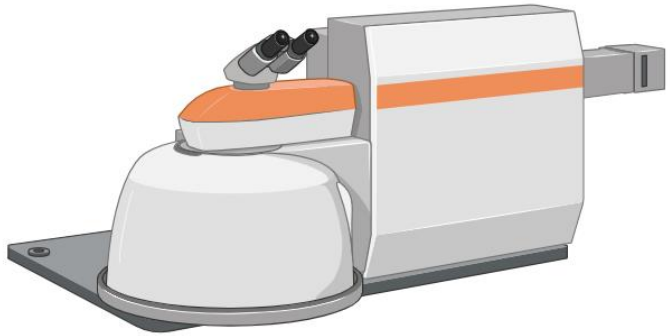
	m	R^2
HPro (IR/Raman)	1,02/1,12	1,00
HBut (IR/Raman)	1,00/1,03	1,00

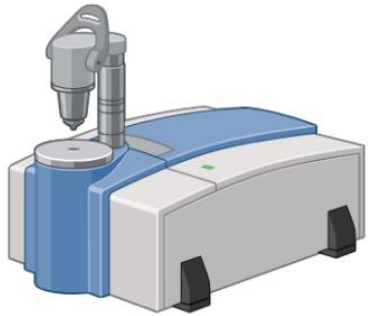
Now, let’s monitor a fermentation using xylose as a substrate

Batch anaerobic fermentation tests x 3

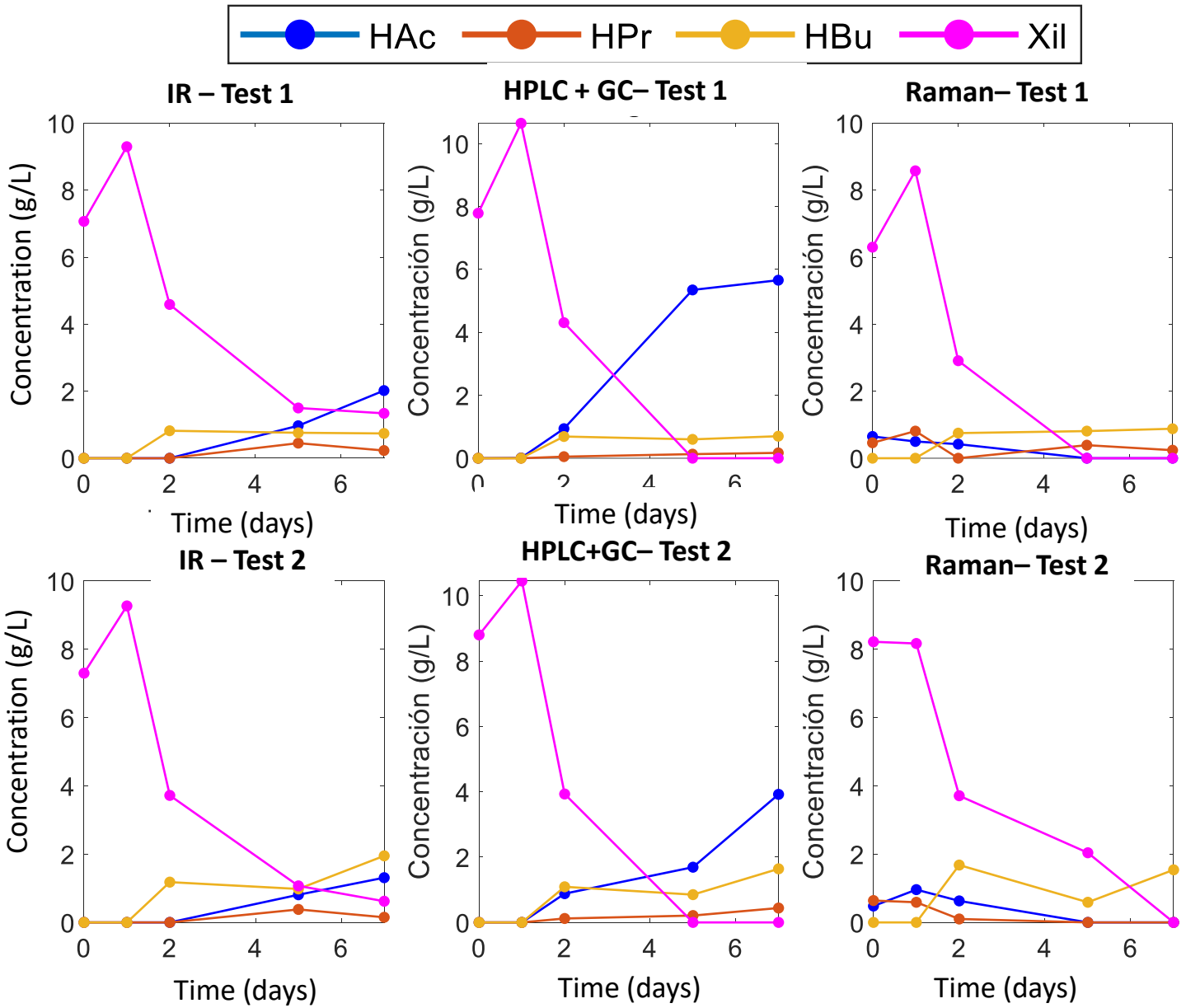


- T=37ºC – bicarbonate buffer + manual correction
- pH≈7
- Xylose 10 g/L
- Sludge form anaerobic fermentation as inoculum
- SIR= 2,00 g SV X/ g SV subs





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

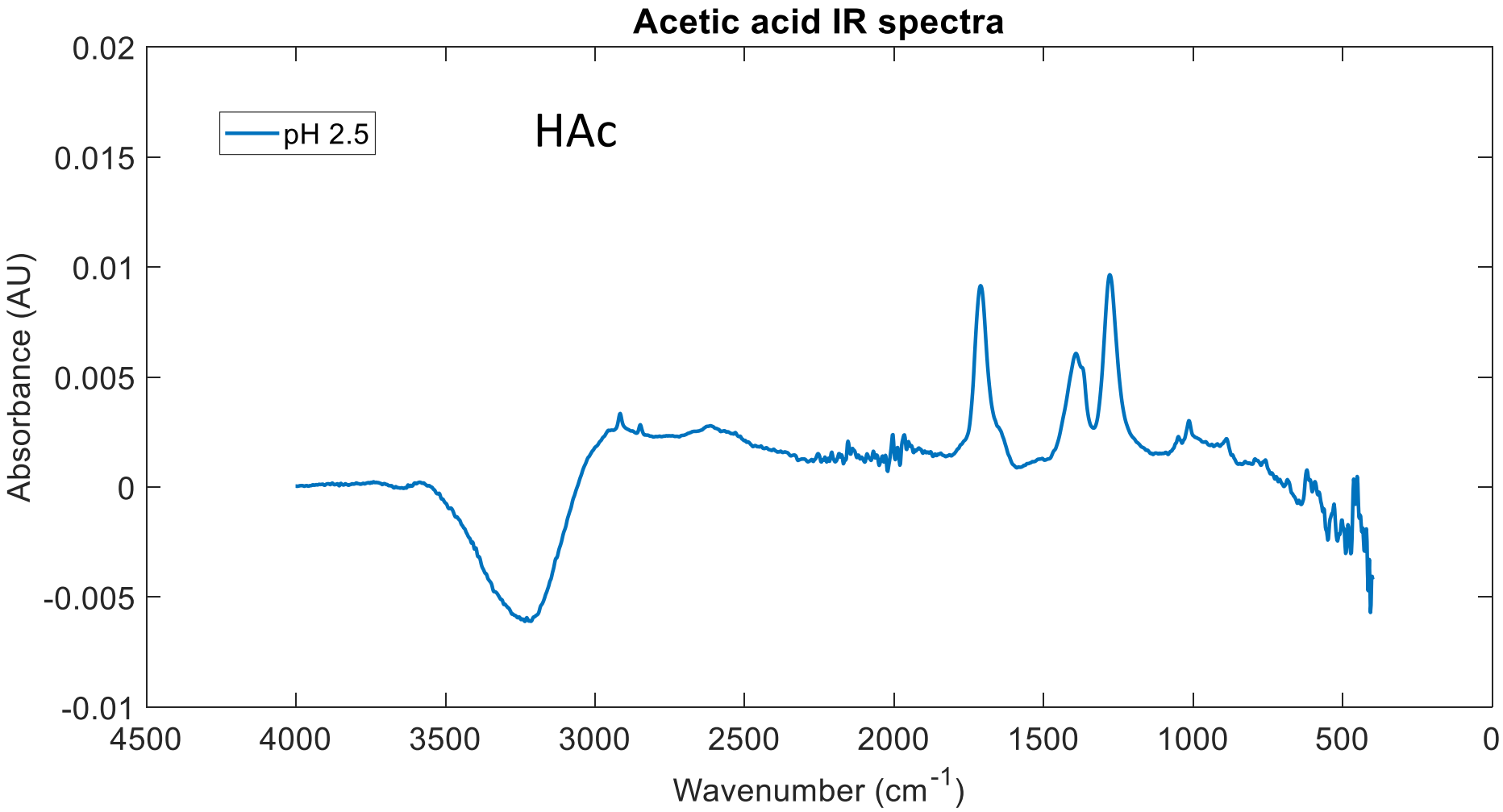
We improve the PLS training and we integrate the data with a model

Better PLS training → Use of independent and spiked fermentation samples

Better PLS training → Control of pH of training samples

Data integration → With a fermentation model through an Extended Kalman Filter

Medium pH plays an important role here



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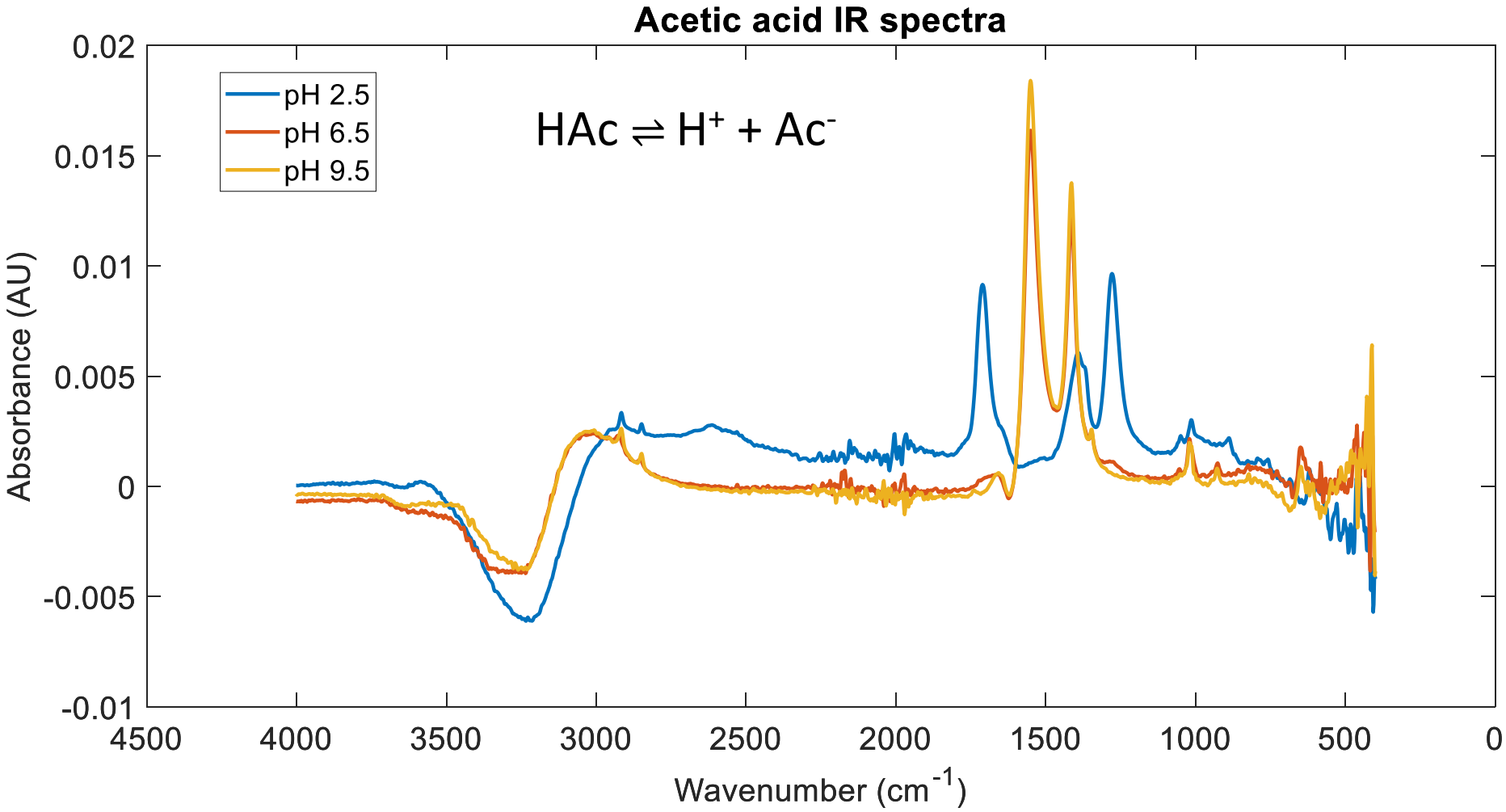
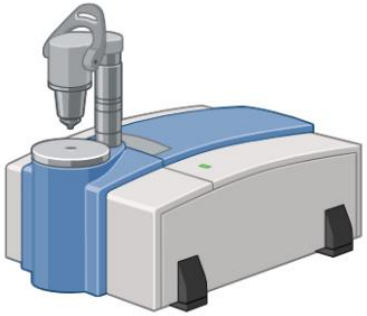
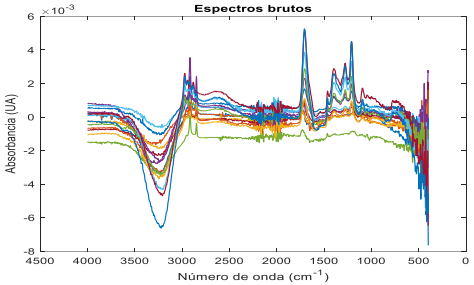


- The peak at 1712 cm^{-1} (C=O stretching vibration of the carboxylic acid group) shifts to 1550 cm^{-1} at higher pH values.
- The peak at 1280 cm^{-1}, (C-O stretching vibration in the carboxylic acid group) shifts to 1415 cm^{-1}

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

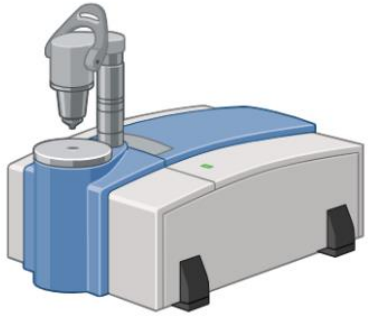


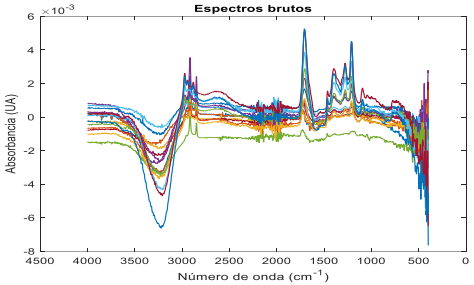


PLS model

HAc, HPro, HBut...
substrate

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



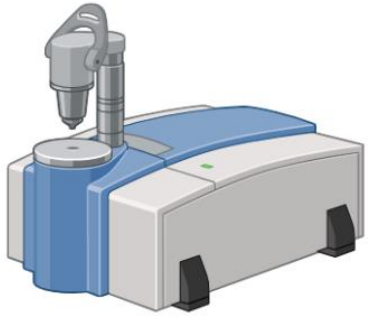


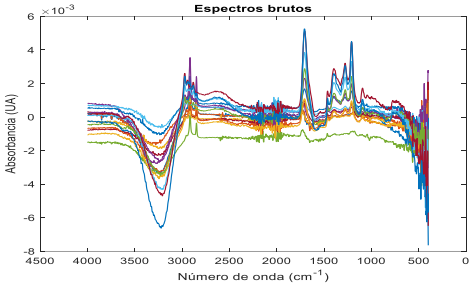
PLS model

HAc, HPro, HBut...
substrate

But the concentrations of acetic, propionic, butyric acid are NOT independent. There are constraints related to mass balances, electron balances, carbon balances…

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

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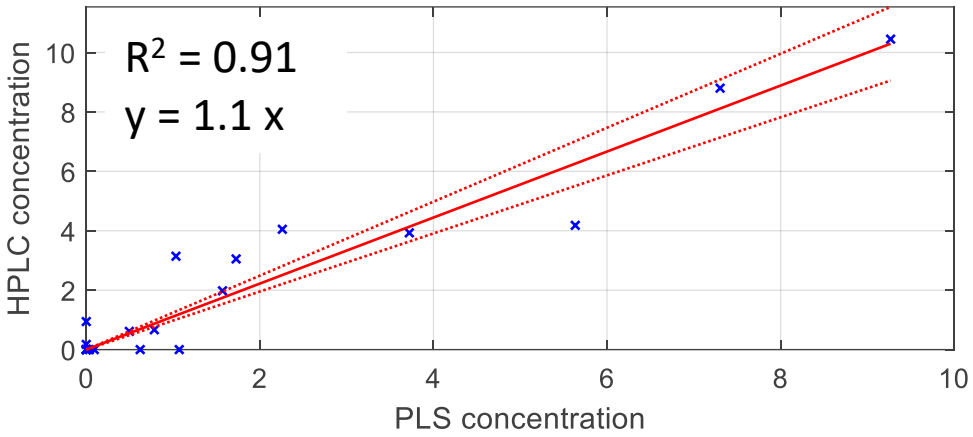
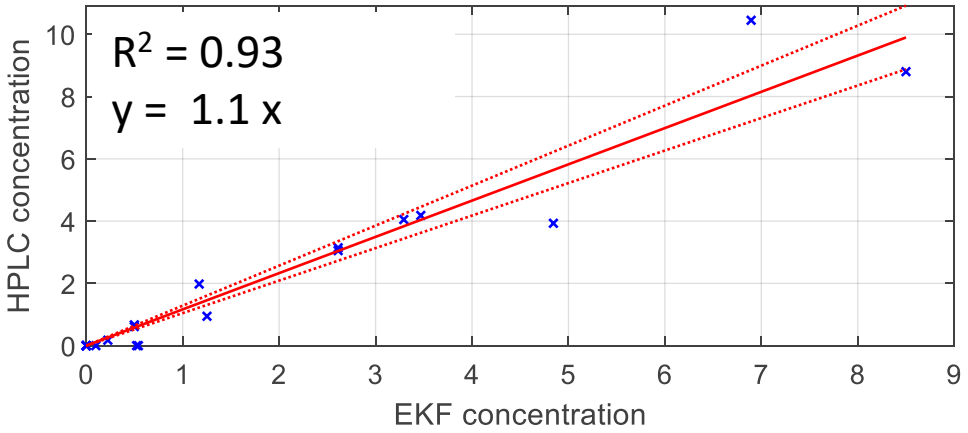
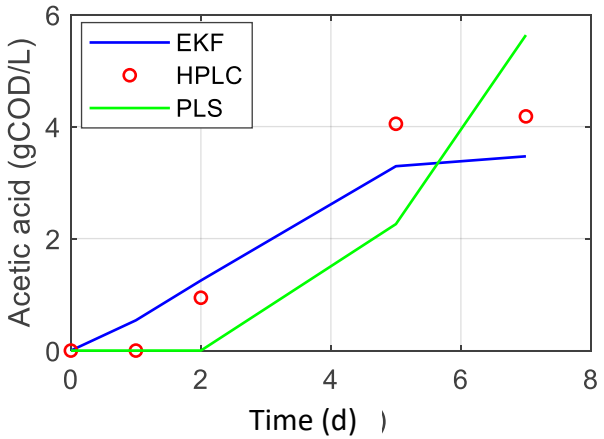
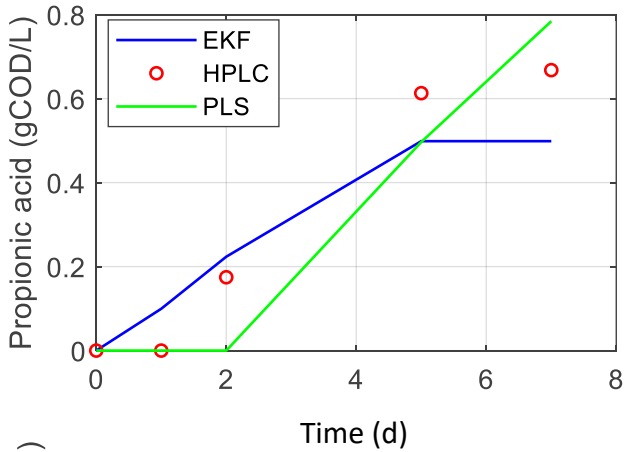
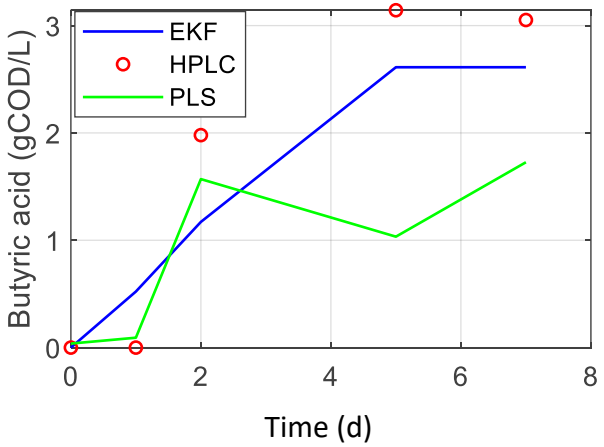
A formulation of an Extended Kalman Filter as a tool to improve the predictive power

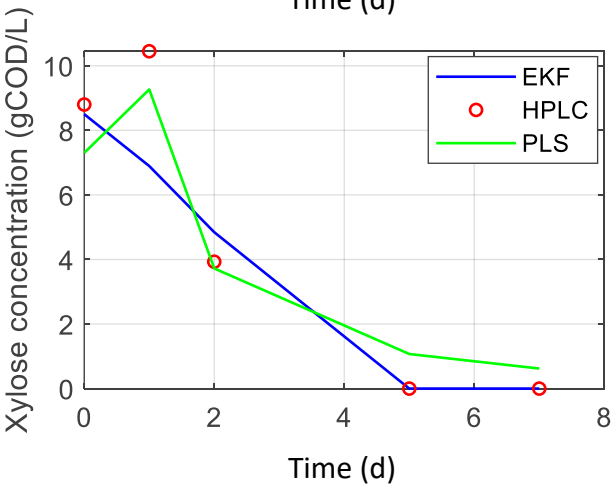












Take home messages

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) The fermentation background and, most importantly, the pH of the fermentation must be taken into account in model training

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

Next steps:
- Integrate ATR fiber for inline measurements
- Monitor continuous fermentation reactor

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Acknowledgements

Manuel de la Fuente

Miguel Bouzas

Juan Cubero

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AGENCIA ESTATAL DE INVESTIGACIÓN

CRETUS Cross-disciplinary Research in Environmental Technologies

BioGroup Group of Environmental Biotechnology

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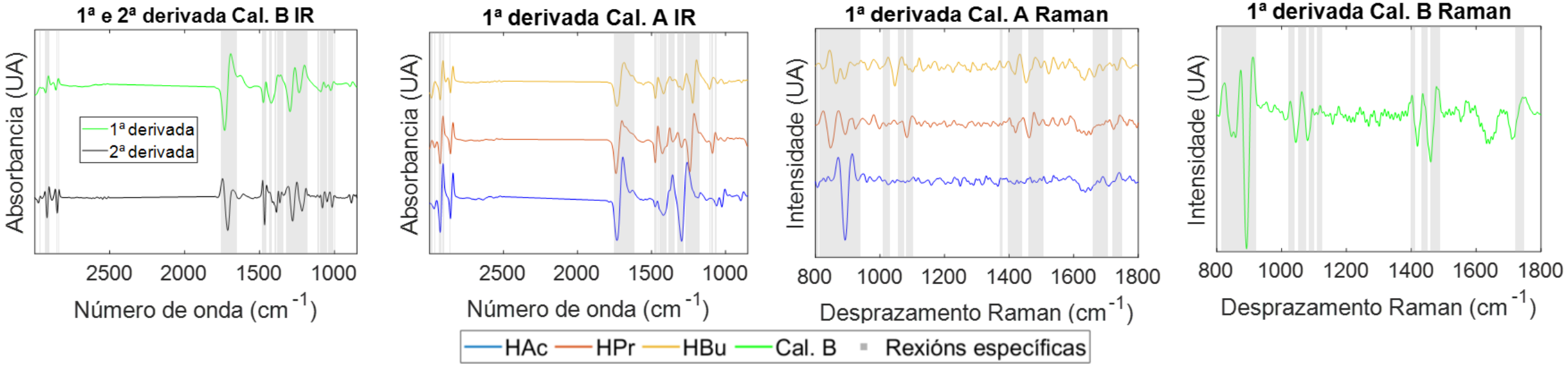
Specific areas in the spectra

Como conseguen os modelos diferenciar os compostos?



-As rexións máis relevantes para os modelos *PLS* son onde se manifestan as bandas dos grupos funcionais caracterizados previamente

-Nos modelos *PLS* das disolucións combinadas adquiren unha maior importancia bandas máis específicas, que son menos intensas