PTR-MS-TOF, ^{1}H NMR and ^{13}C MAS NMR for the determination of organic compounds produced by fibre degradation in the MELiSSA project

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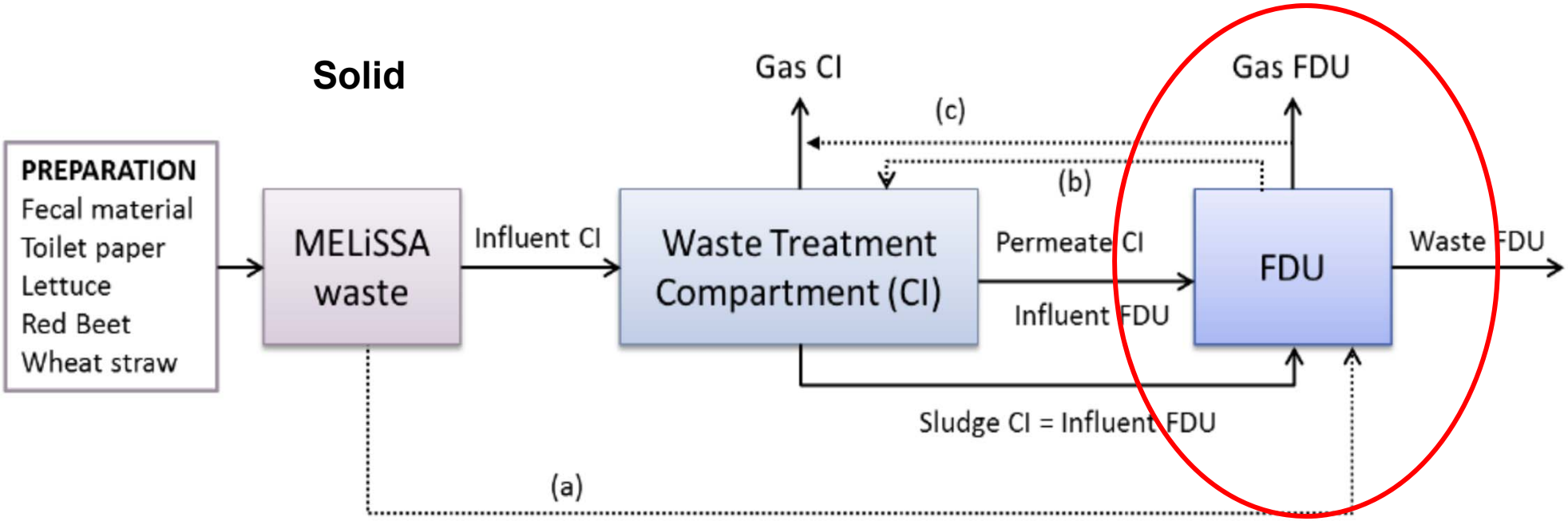
The aim of this presentation is to show the type of information that ^{1}H NMR, ^{13}C MAS NMR and PTR-MS can provide on the composition of different organic compounds in fibre-containing wastes, and in the liquid and gas solutions obtained from their biological and chemical degradation.

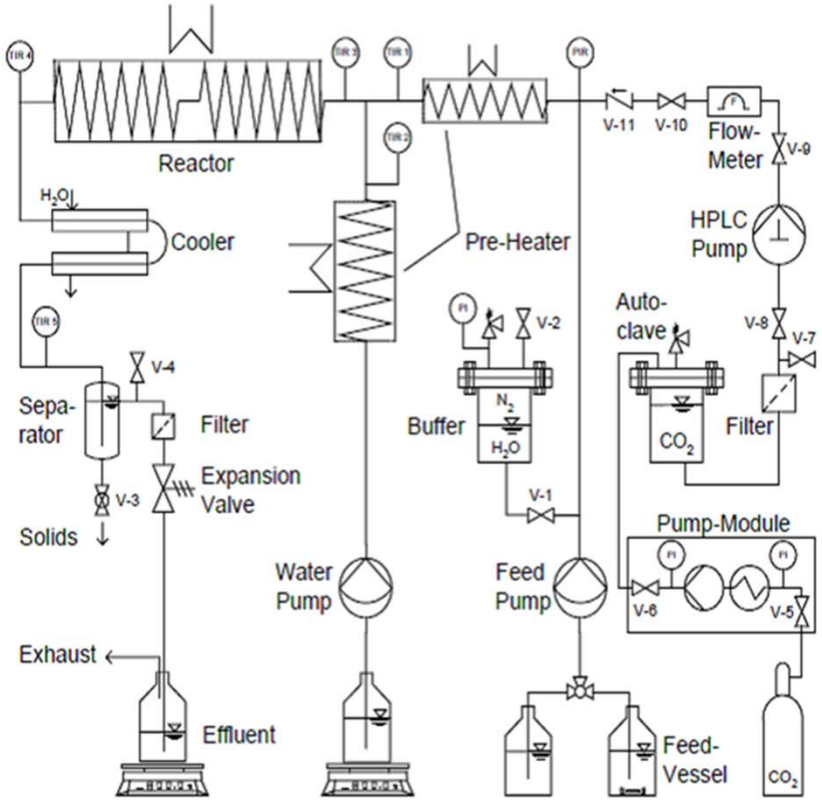
These techniques were used in the proposal aimed at Developing a Fibre Degradation Unit (FDU) able to convert liquid and sludge wastes from the C1 biological reactor of the MELissa system, activated with mixed inoculum, into gases (mainly CO2 and H2) with an efficiency higher than 99%.



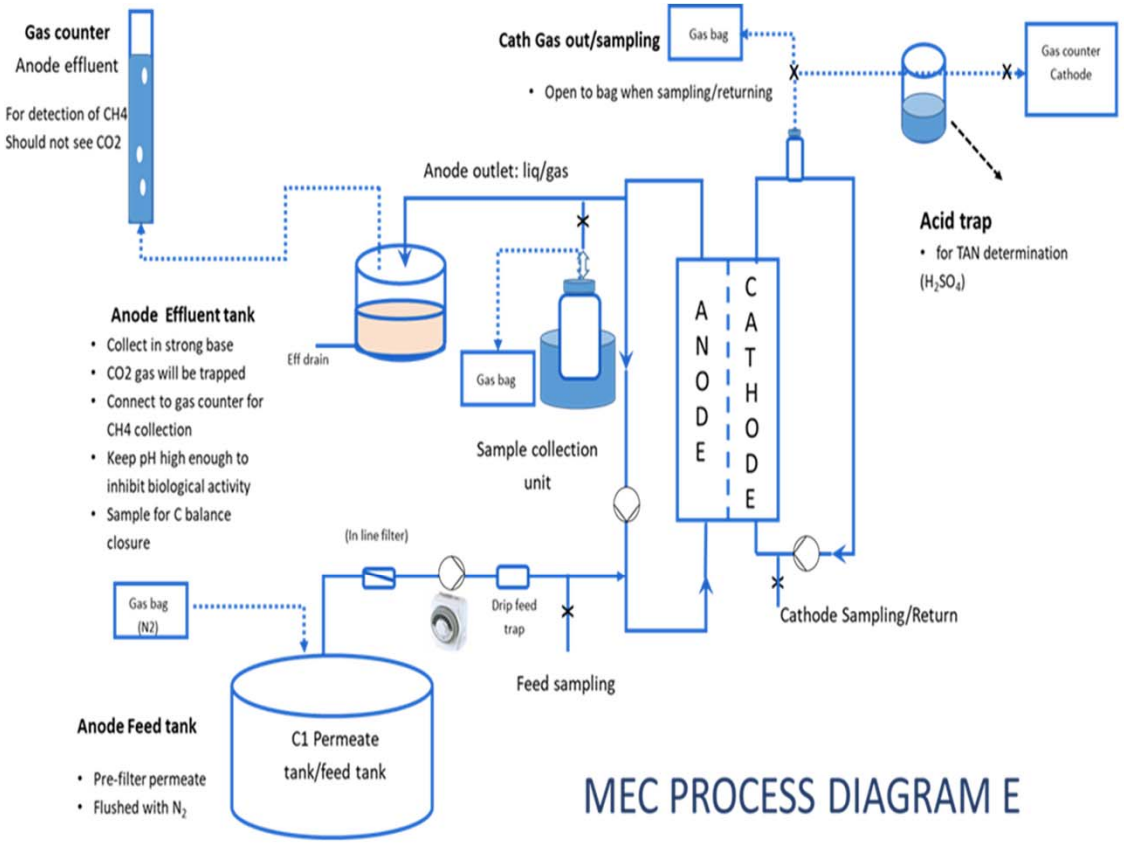
The activity was co-ordinated by the Center for Microbial Ecology and Technology (CMET) of the University of Gent, where IMC-CNR acted as one of the sub-contractors.

The **FDU was composed by 2 units,** one for the gasification of residual **SLUDGE using a Supercritical Water Oxidation unit (SCWO),** and the other for the degradation of the **PERMEATE liquid by a Microbial Electrolytic Cell (MEC)** converting Volatile Fatty acids into CO_2 and H_2. The technical description of the FDU units and the performances obtained with them will be provided in the presentation by **Amanda Luther et al..**

SCWO



MEC

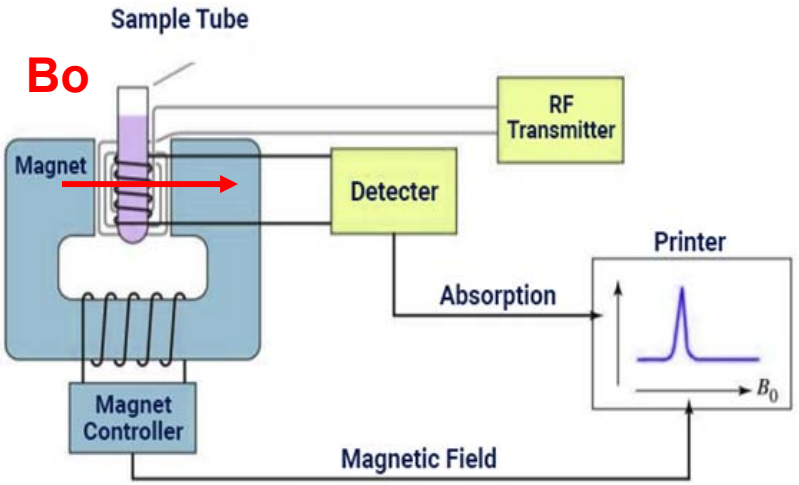


Here we focus on the experience we gained though the analysis of the solid, liquid and gaseous samples that were provided to us by CMET of the University of Gent.

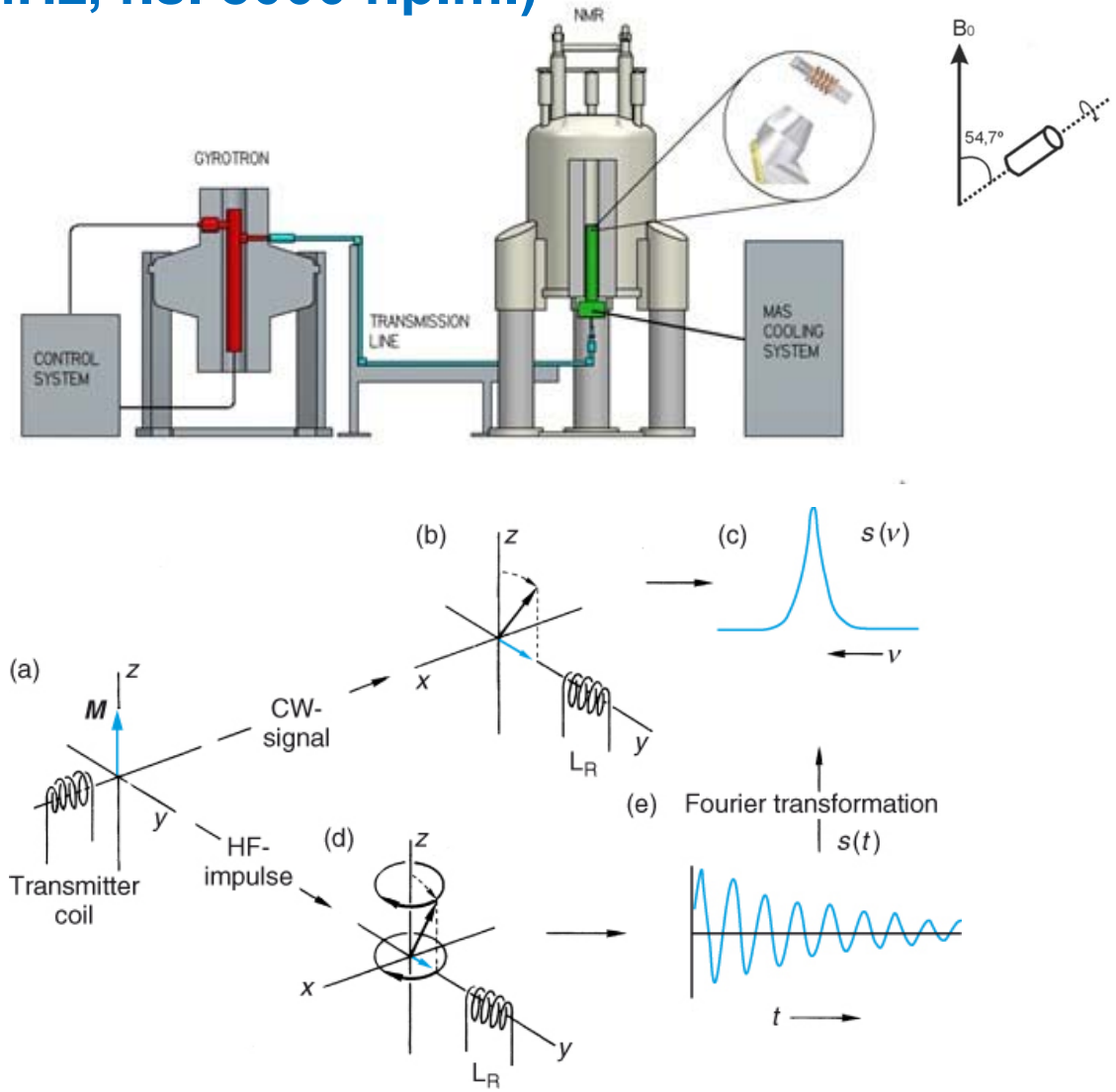
Some of the results certainly open new possibilities to replace some of the most time consumimg analytical methods commonly used to assess the fibre degradation with biological and chemical processes, and the biogas emission from them.

We start with those used in the analysis of solid and liquid samples, where NMR spectroscopy was used, in combination with other techniques.

Liquid solutions were analyzed by ^{1}H NMR (600 MHz, 90°)

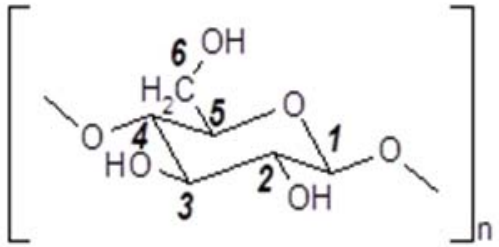


Solids were analyzed by ^{13}C Magic Angle Spinning (MAS) NMR spectrometry (400 MHz, r.s. 8000 r.p.m.)



The signal is generated by nuclear spin transitions of ^{1}H and ^{13}C atoms induced by High Frequency Electromagnetic pulses to the whole material kept in a high Magnetic field (Bo).

^{13}C MAS NMR spectra of solids



C=O
Lignin
Hemicellulose
Cellulose

CELLULOSE
a=amorphous
c=crystal

OCH3
Lignin

LIGNIN

C2, C3, C5

Amino acids

C4a

C6c

C1

C6a

C4c

180 160 140 120 100 80 60 40 ppm

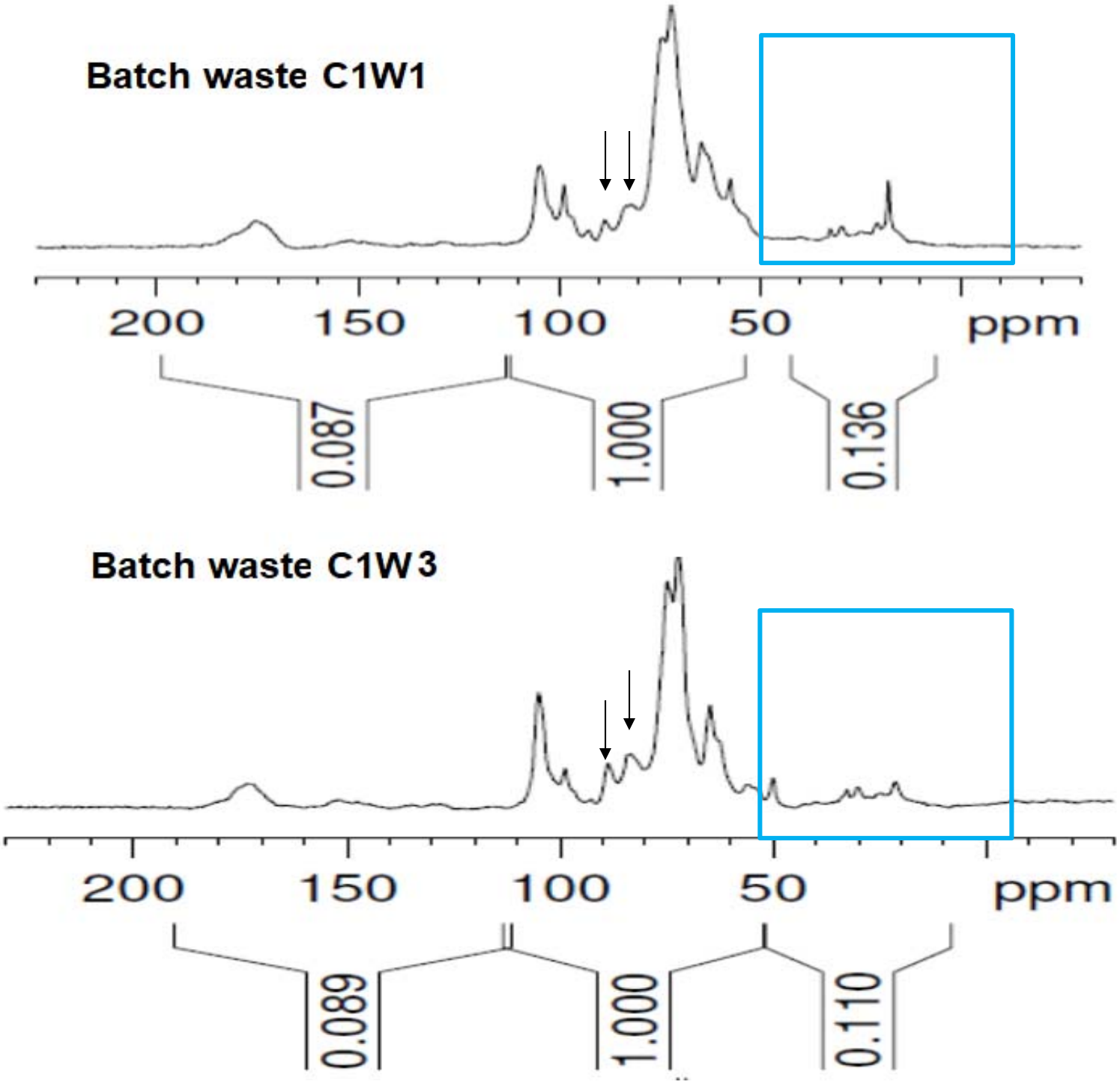
MELISSA WASTE SAMPLE

TOILET PAPER

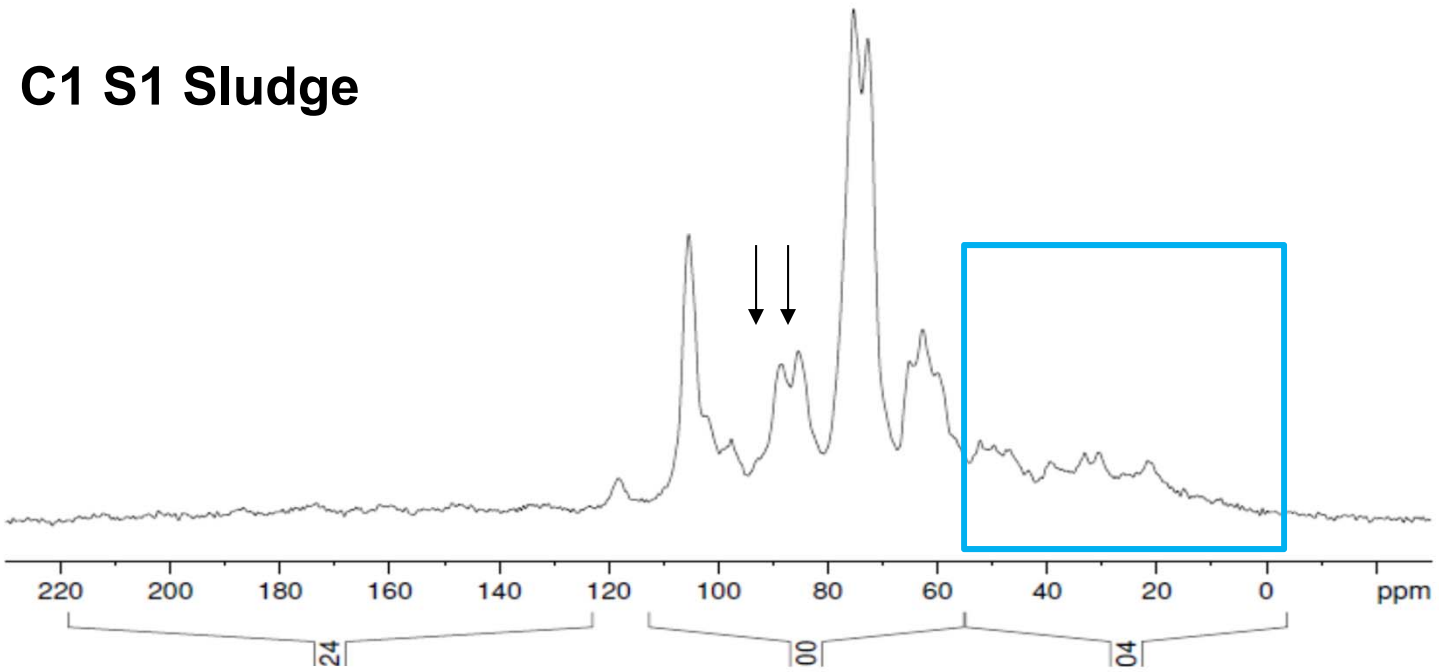
180 160 140 120 100 80 60 40 ppm

WOOD

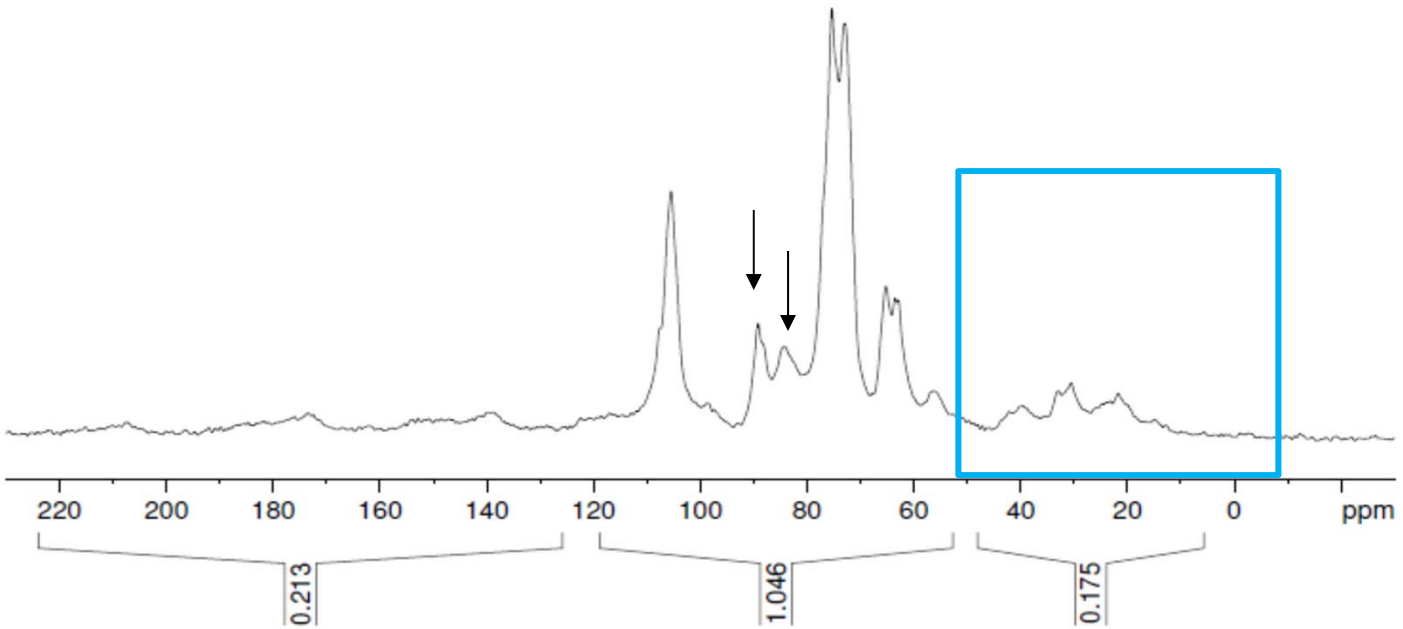
180 160 140 120 100 80 60 40 ppm


Batch waste C1W1
200
150
100
50
ppm
0.087
1.000
0.136
Batch waste C1W3
200
150
100
50
ppm
0.089
1.000
0.110

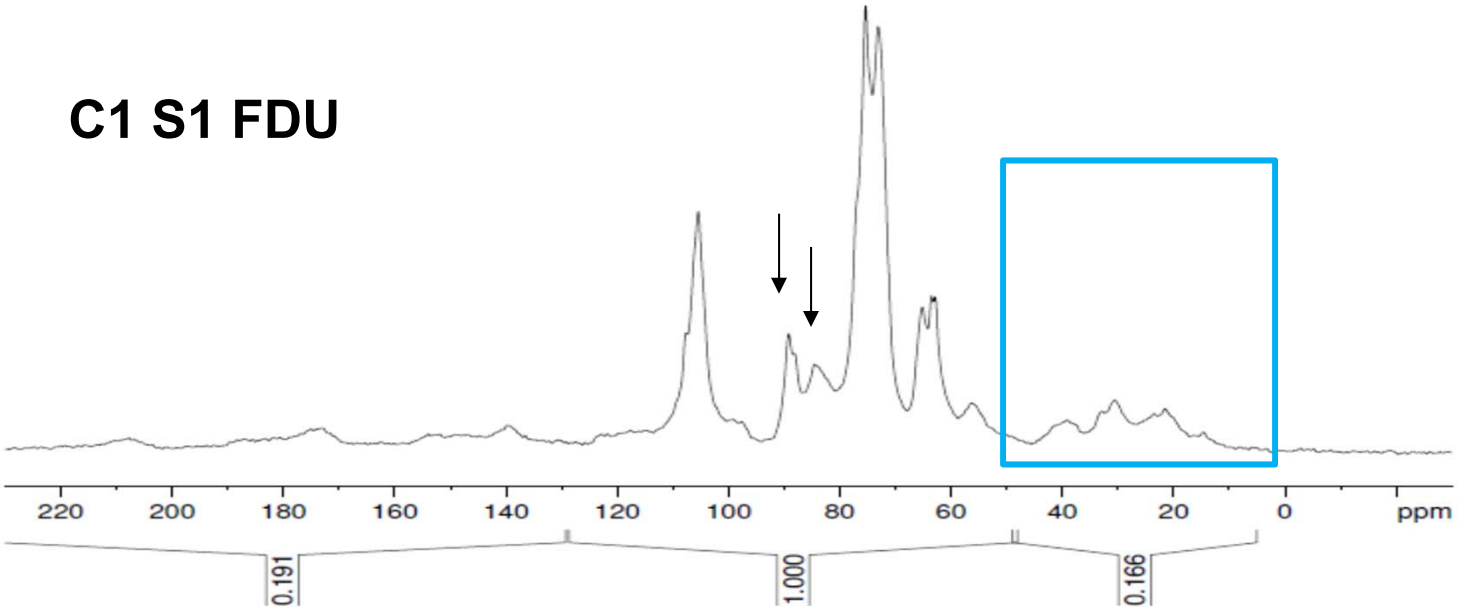
C1 S1 Sludge



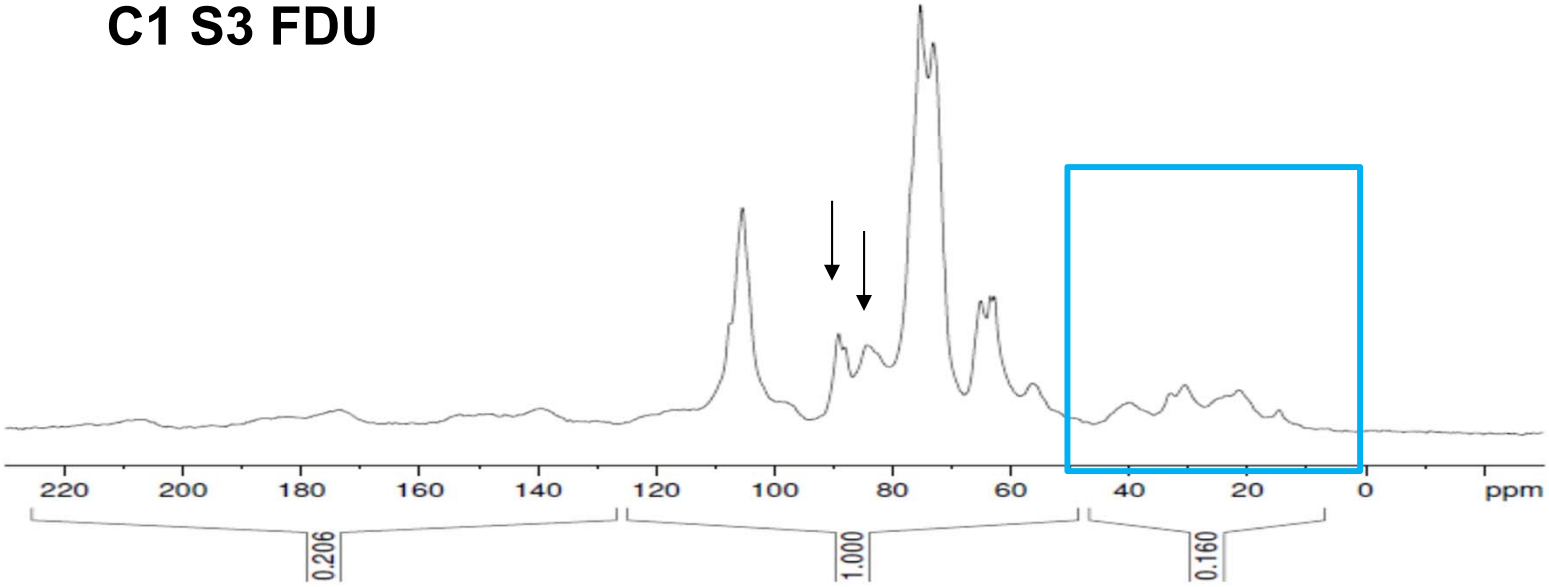
C1 S3 Sludge



C1 S1 FDU



C1 S3 FDU



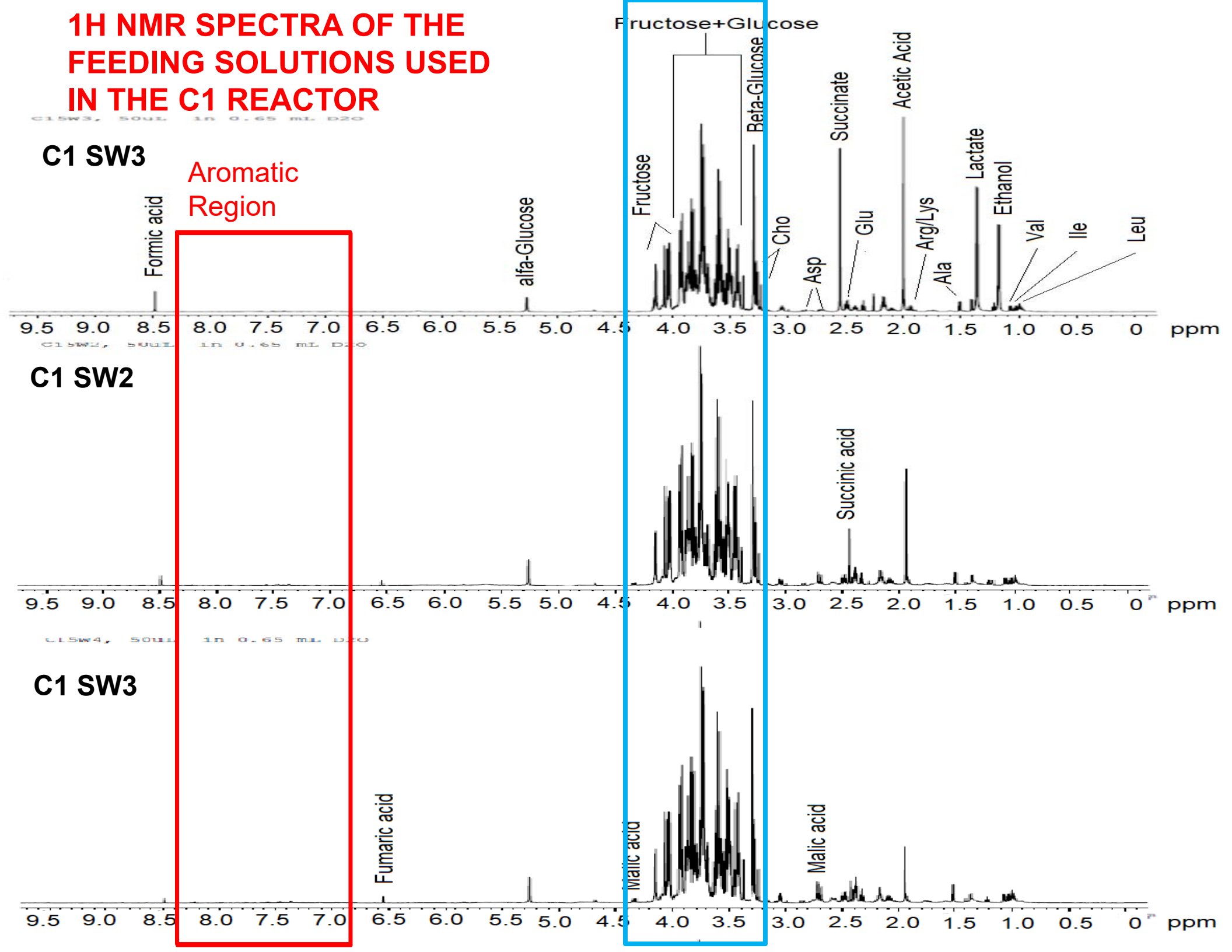
The data show that ^{13}C NMR provides a comprehensive view of the various components, including amino acids, and of the structural changes in the cellulose structure.

For quantitation purposes, reference materials for cellulose, hemicellulose and lignin are required with a degree of disorder close to that of the sample.

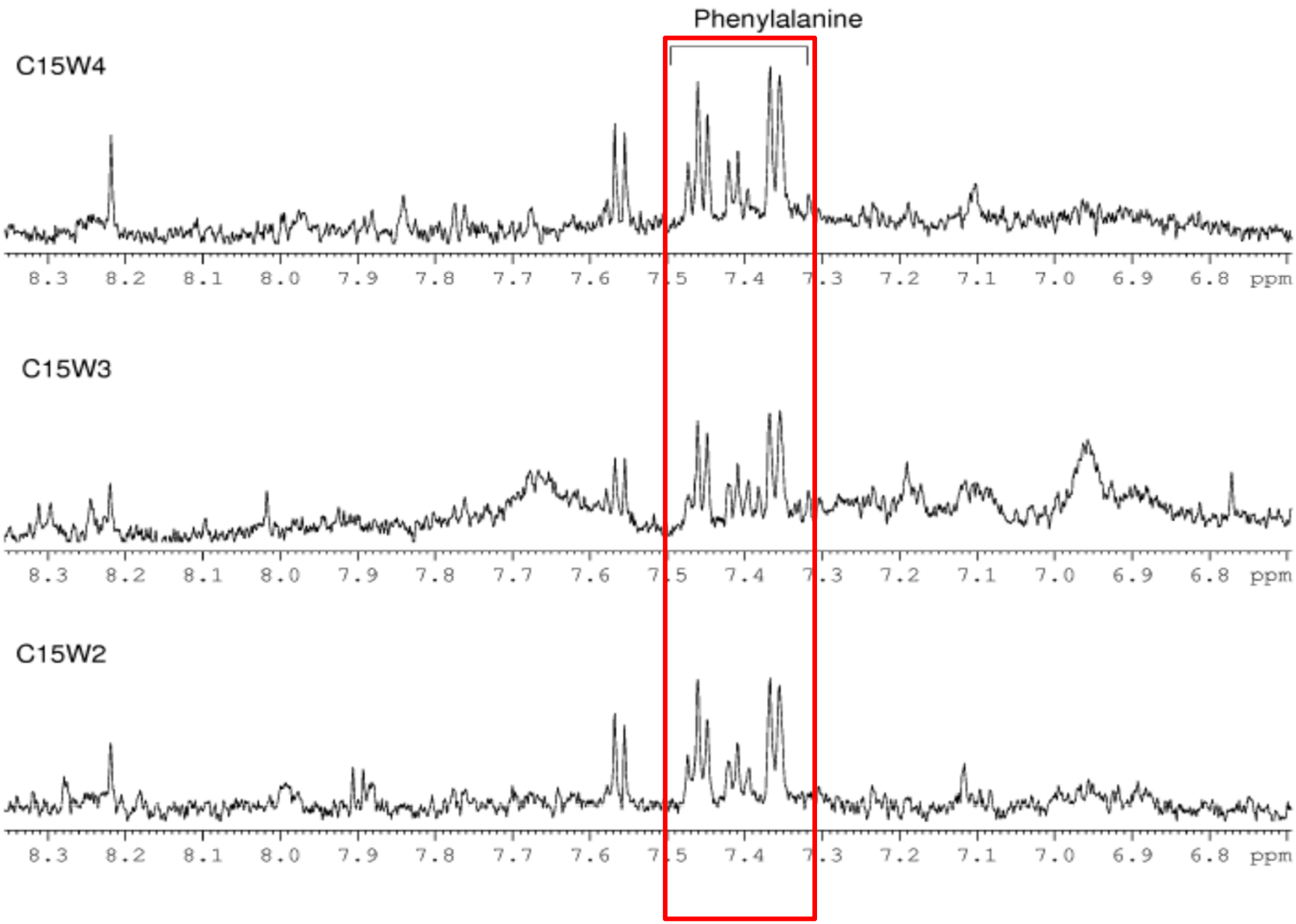
We are now searching for them.

If succeful, the method can replace the chemical treatments for the determinations of the major and minor components in dried samples, as it requires about 2 hours for a complete aquisition.

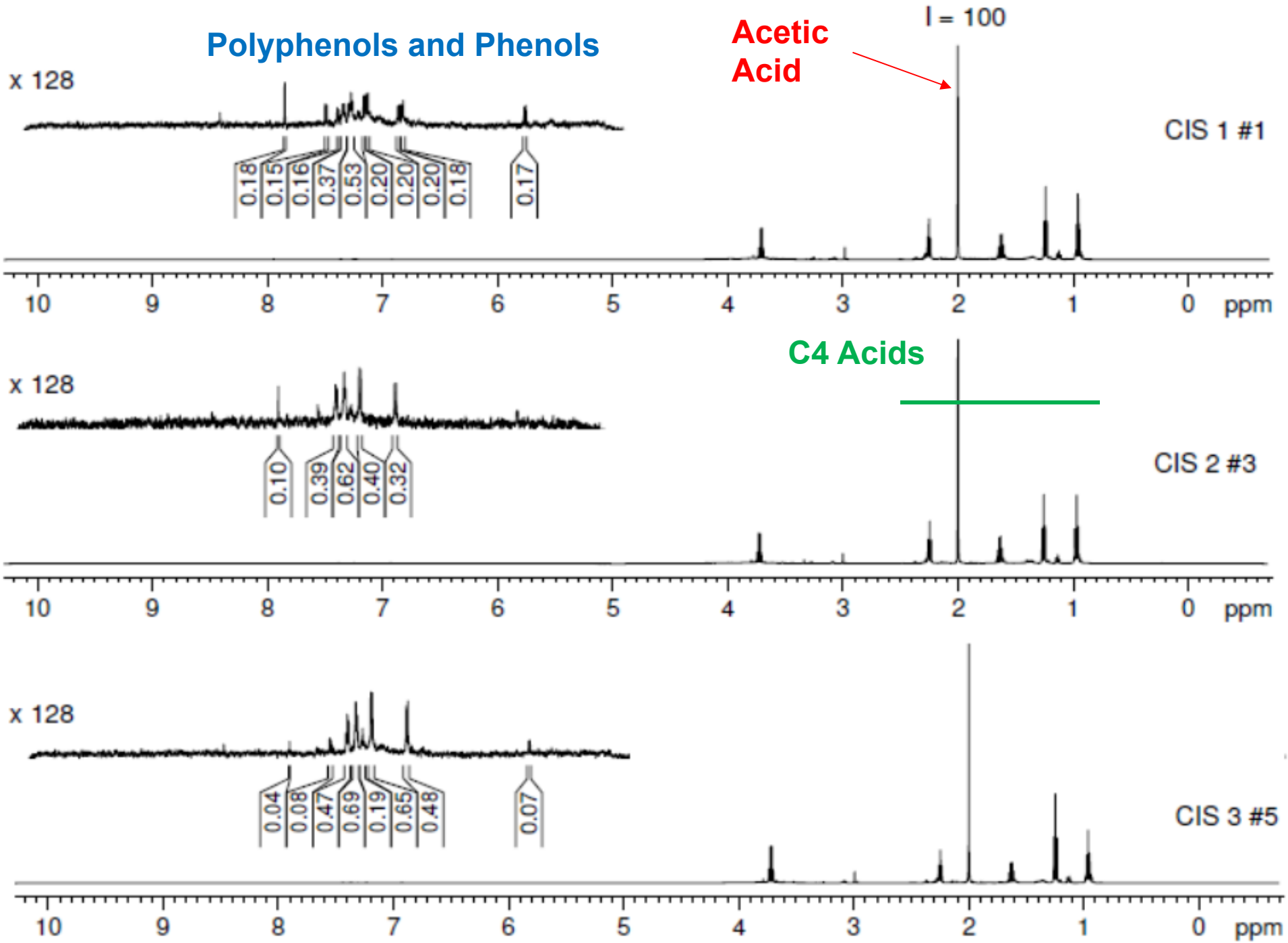
1H NMR SPECTRA OF THE FEEDING SOLUTIONS USED IN THE C1 REACTOR



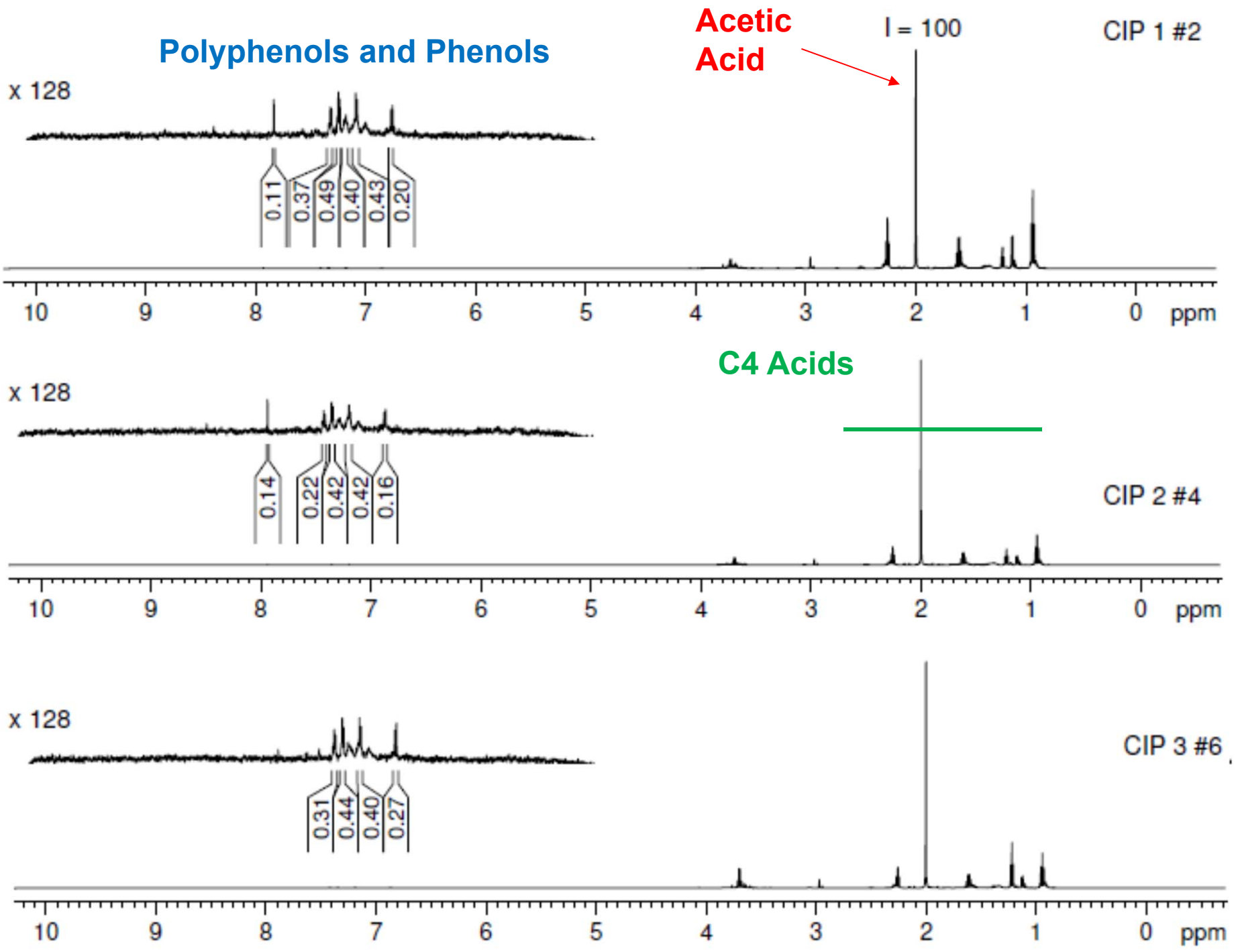
Expanded view of the Aromatic Region. Intensity x 128

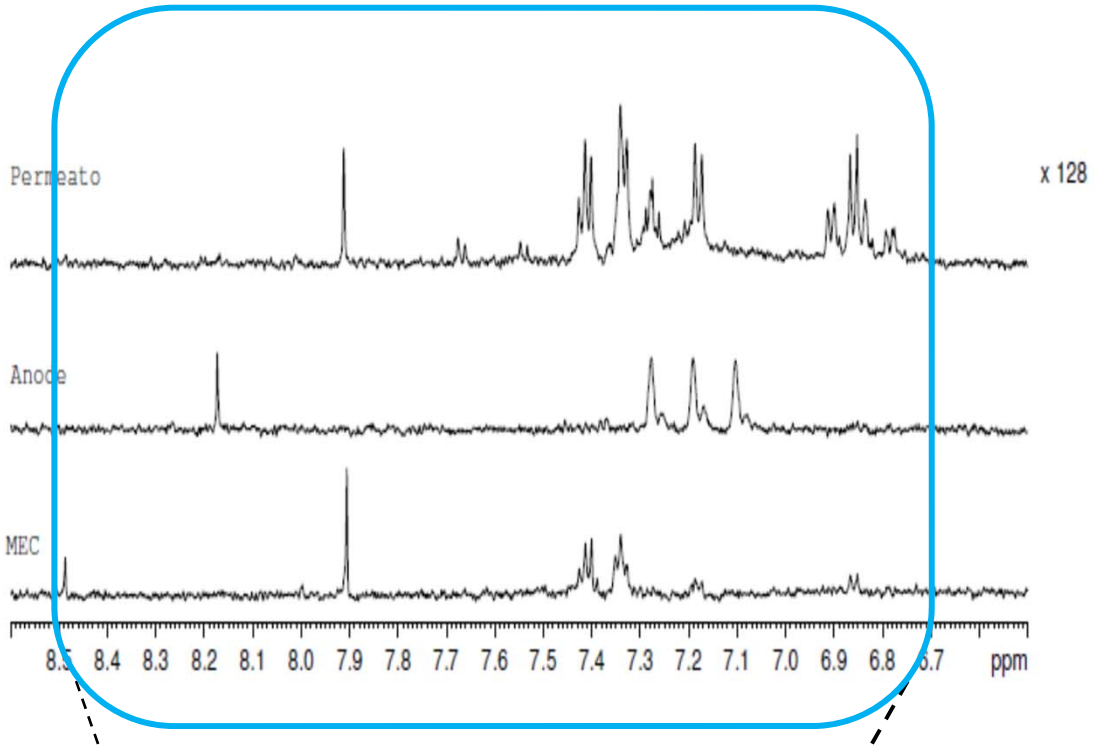


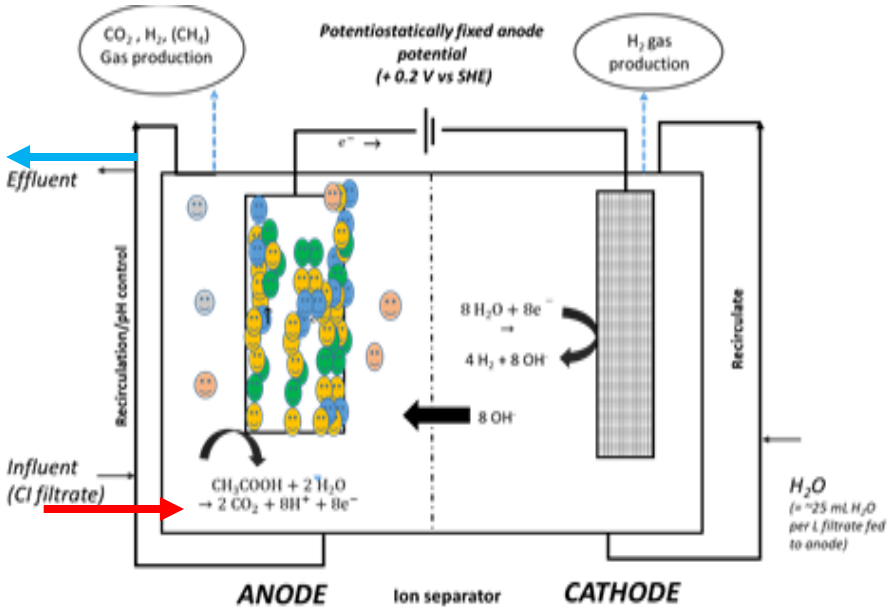
FILTRATE SOLUTIONS FROM THE C1 REACTOR



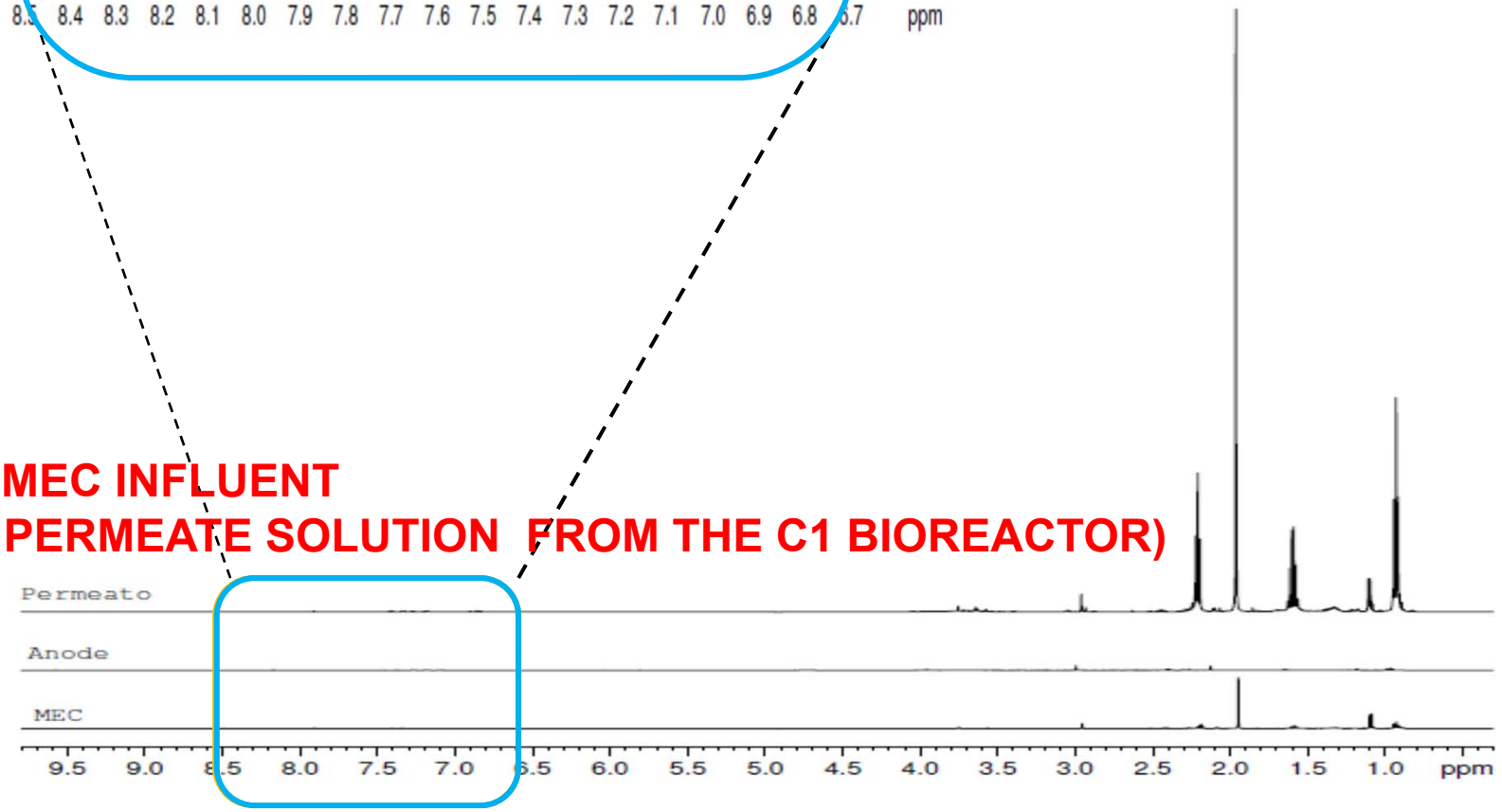
FILTRATE+PERMEATE SOLUTIONS FROM THE C1 REACTOR



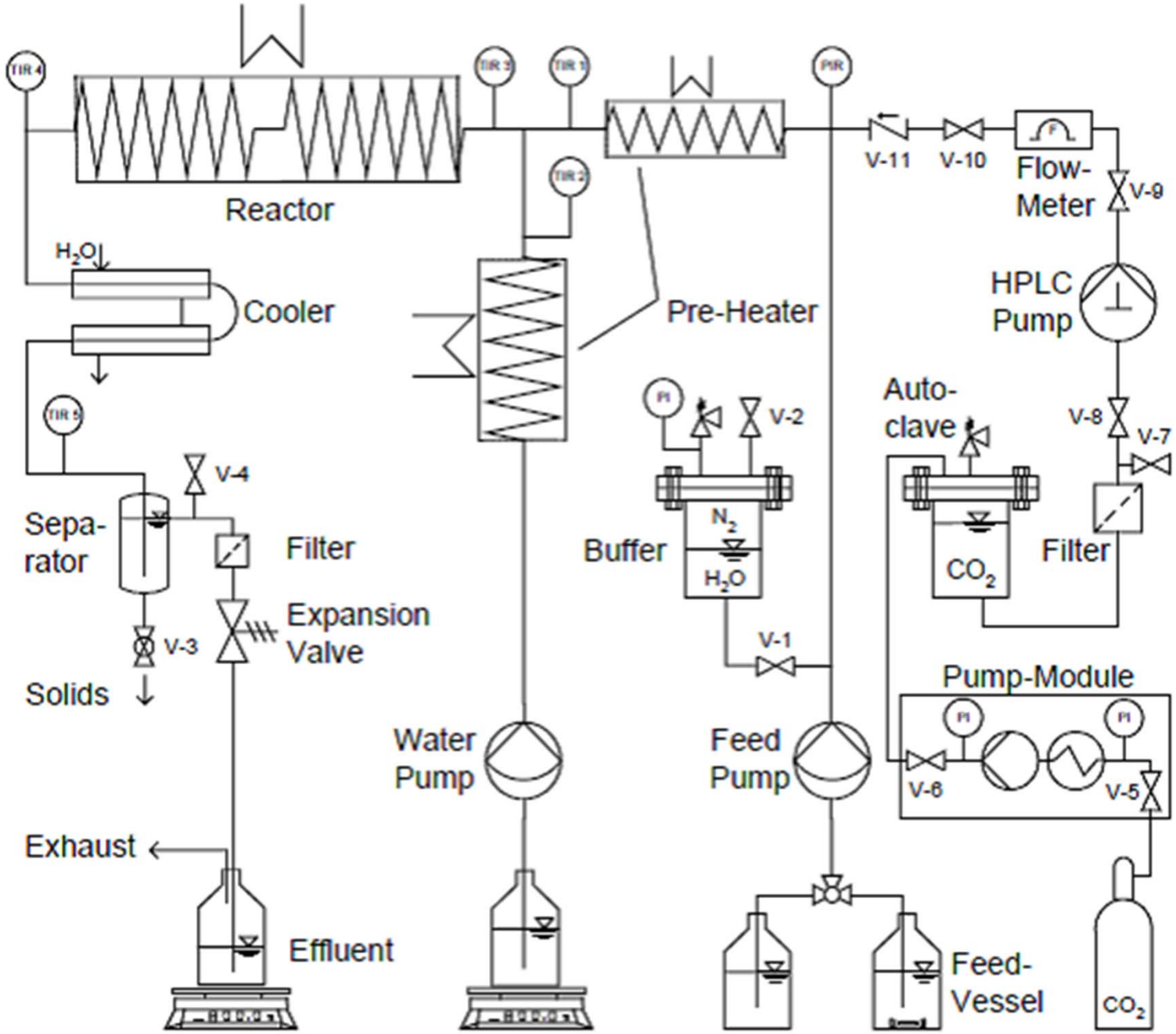




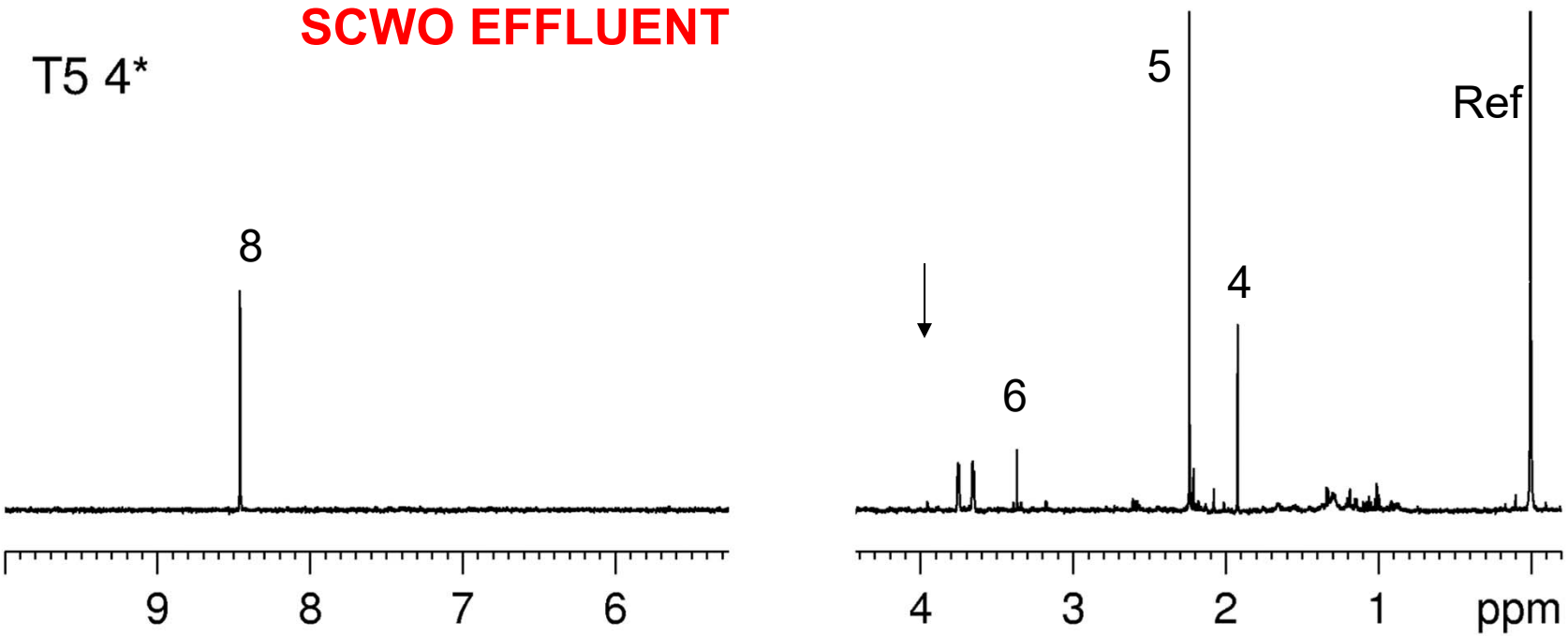
MEC INFLUENT
(PERMEATE SOLUTION FROM THE C1 BIOREACTOR)

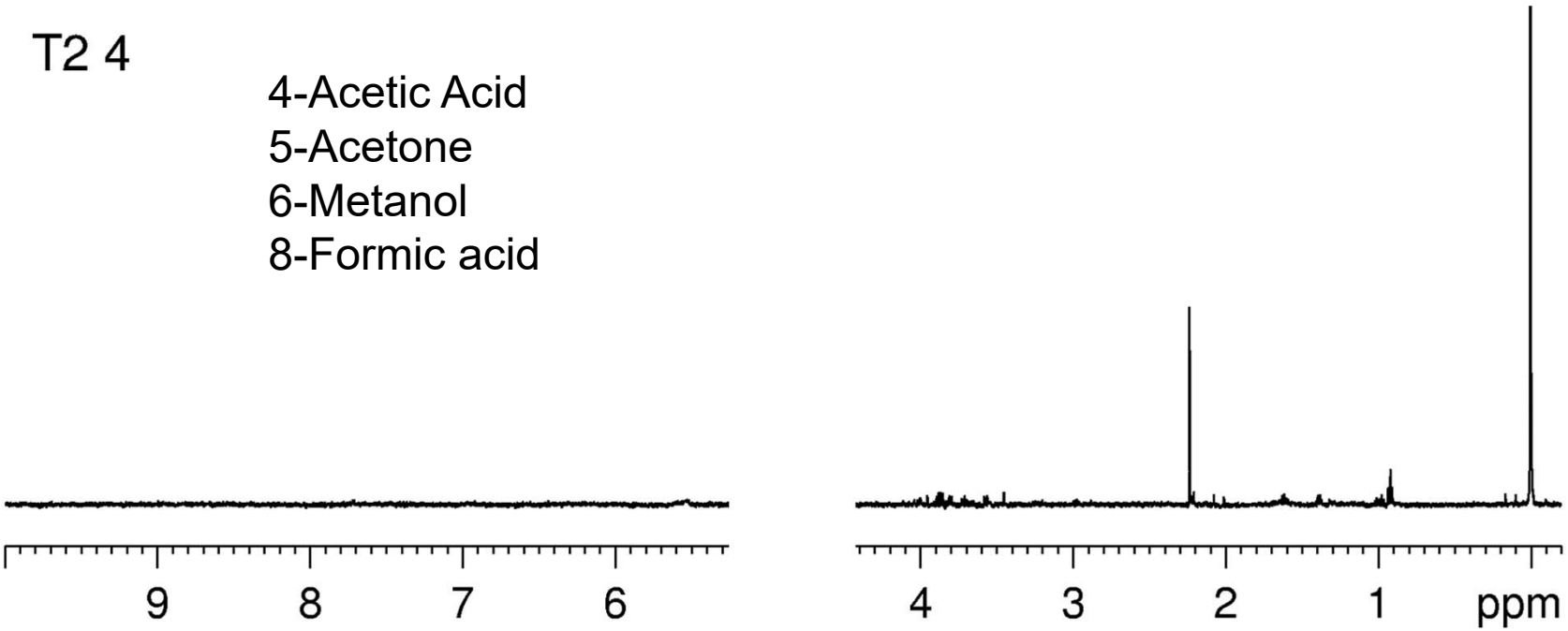


LIQUID MEC EFFLUENTS


TIR 4
TIR 3
TIR 1
PIR
Reactor
V-11
V-10
Flow-
Meter
V-9
TIR 2
H_2O
Cooler
Pre-Heater
HPLC
Pump
TIR 5
PI
V-2
Auto-
clave
V-8
V-7
V-4
N_2
Sepa-
rator
Filter
Buffer
H_2O
CO_2
Filter
Expansion
Valve
V-3
V-1
Pump-Module
Solids
Water
Pump
Feed
Pump
V-6
V-5
Exhaust
Effluent
Feed-
Vessel
CO_2

SCWO EFFLUENT





Data show that ^{1}H NMR provides also a comprehensive view of the organic composition of the liquids obtained from the various steps of the fibre degradion in the C1 and FDU units.

By knowing the compounds present in the various solutions, a proper selection can be made to see whatelse is present in them.

This technique helps to discard other data, that for some reasons were affected by some errors, such as sample contamination, that occurred once during the GC-FID and GC-MS determinations of a couple of solutions.

PROTON-TRANSFER MASS SPECTROMETRY WITH A TIME OF FLIGHT DETECTOR (PTR-MS-TOF) FOR THE DETECTION OF VOC_S IN GAS AND LIQUID SOLUTIONS

PTR-MS exploits the ion-molecular reaction between H_3O^+ and a chemical species R to generate ions in the MS source:

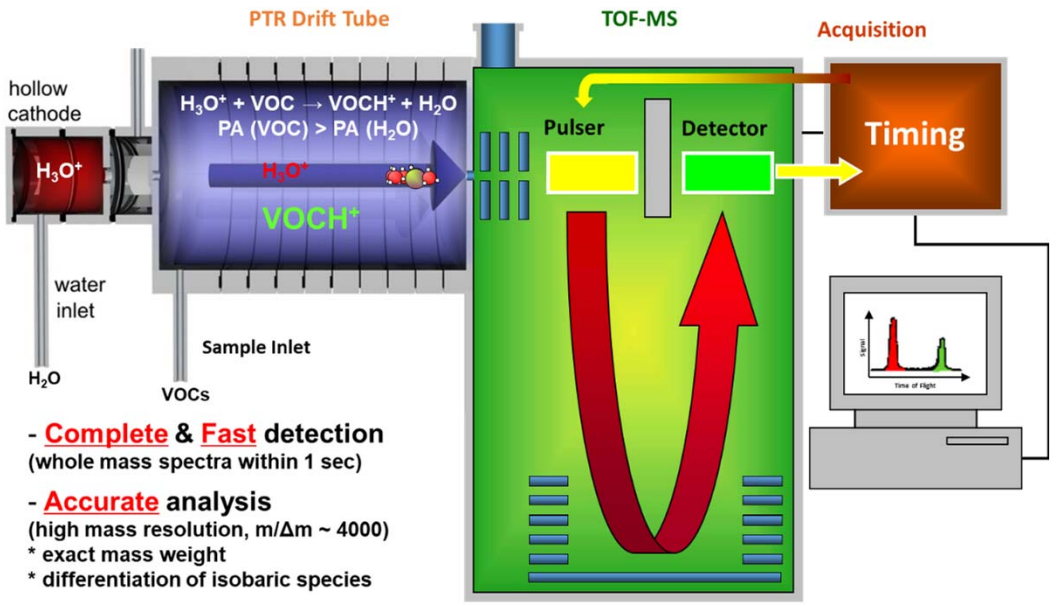
$$H_3O^+ + R \rightarrow RH^+ + H_2O$$

The reaction occurs in the MS source, only if the proton affinity (PA) of R is higher than 165 Kcal/mol, but this occurs for all hydrocarbons, except alkanes and halogen containing compounds.

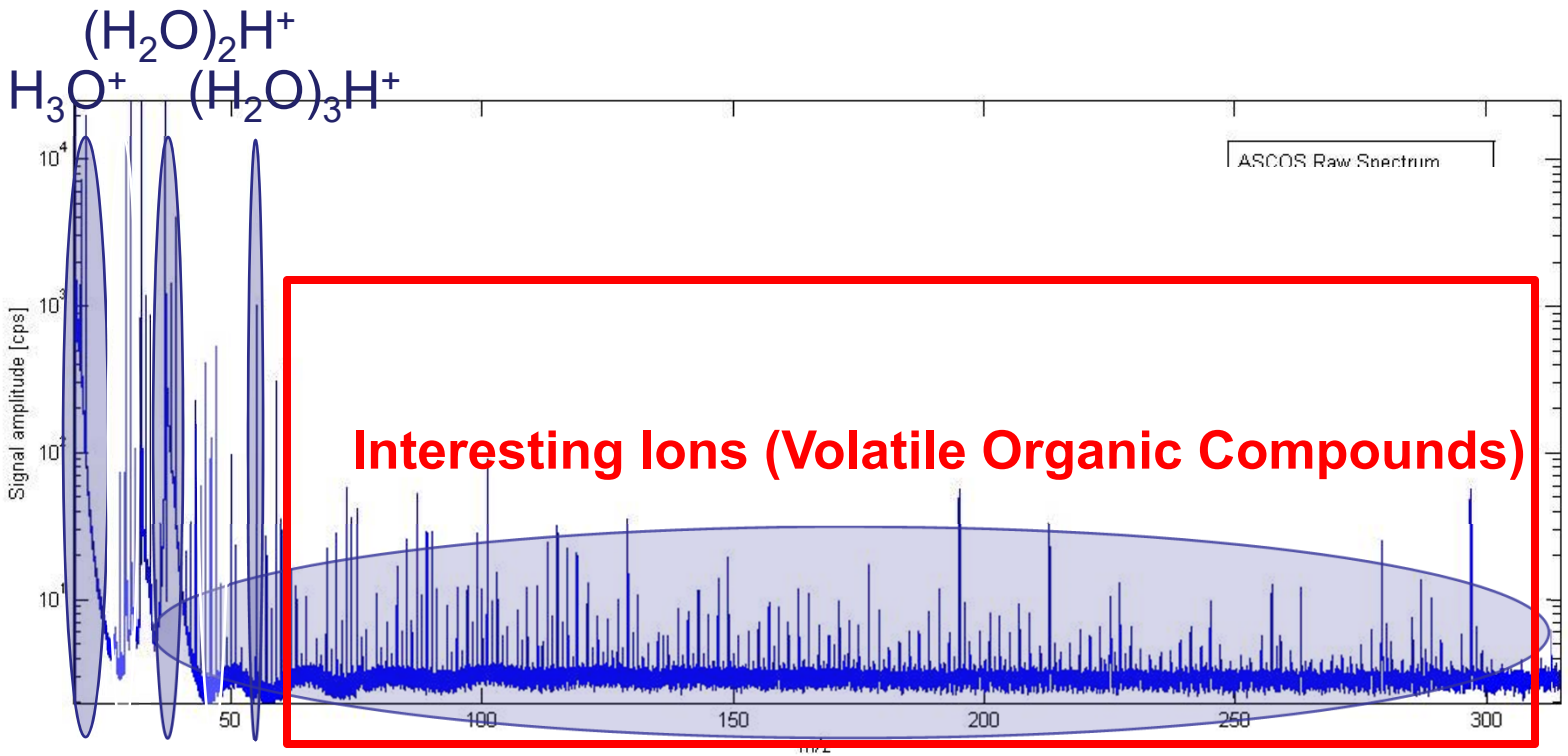
The reaction is also possible with S and N containing organic and inorganic compounds, including H_2S and HCN.

Thanks to the high resolution afforded by the TOF detector, PTR-MS allows to detect compounds differing by 0.03 Da of mass units, in a time of seconds. The detection limits are such that compounds in the order 10 pptv can be detected.

Proton Transfer Reaction Time-Of-Flight Mass Spectrometer (PTR-TOF-MS)



Primary Ions



In the following, the performance of the HR PTR-TOFMS is demonstrated by separation of isobars. With an example protonated ions of **glyoxal** ($C_2H_2O_2.H^+$) and **acetone** ($C_3H_6O.H^+$), figure (3) demonstrates, that two compounds can be distinguished.

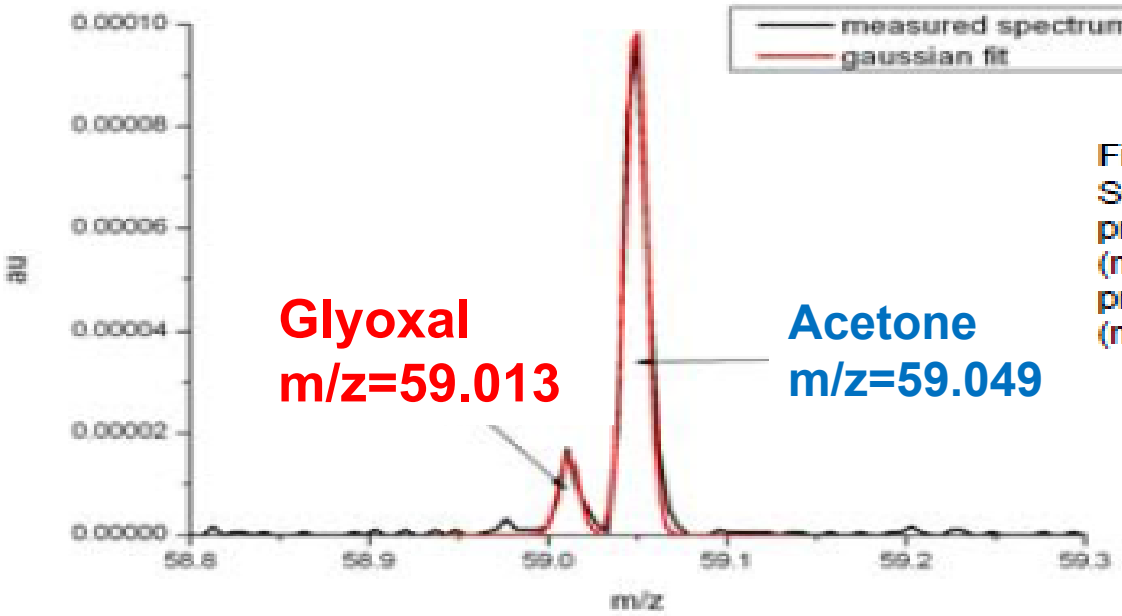


Figure (3): Separation of protonated glyoxal (m/z=59.013) and protonated acetone (m/z=59.049)

Figure (4) shows three peaks at m/z=47 (protonated **formic acid**, $N_2H_3O^+$ and protonated **ethanol**), which can be separated. Identification of the mass peaks (**empirical formula**) is done by their respective exact mass and verified by their **isotopic patterns**.

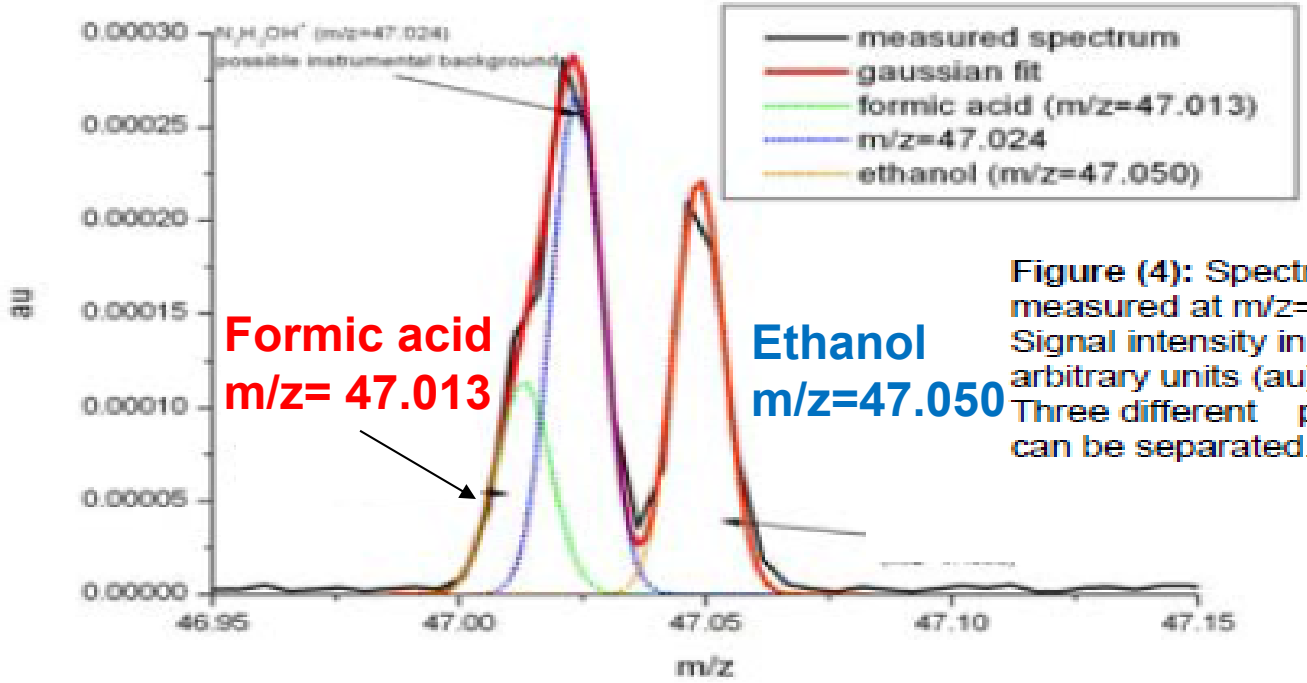
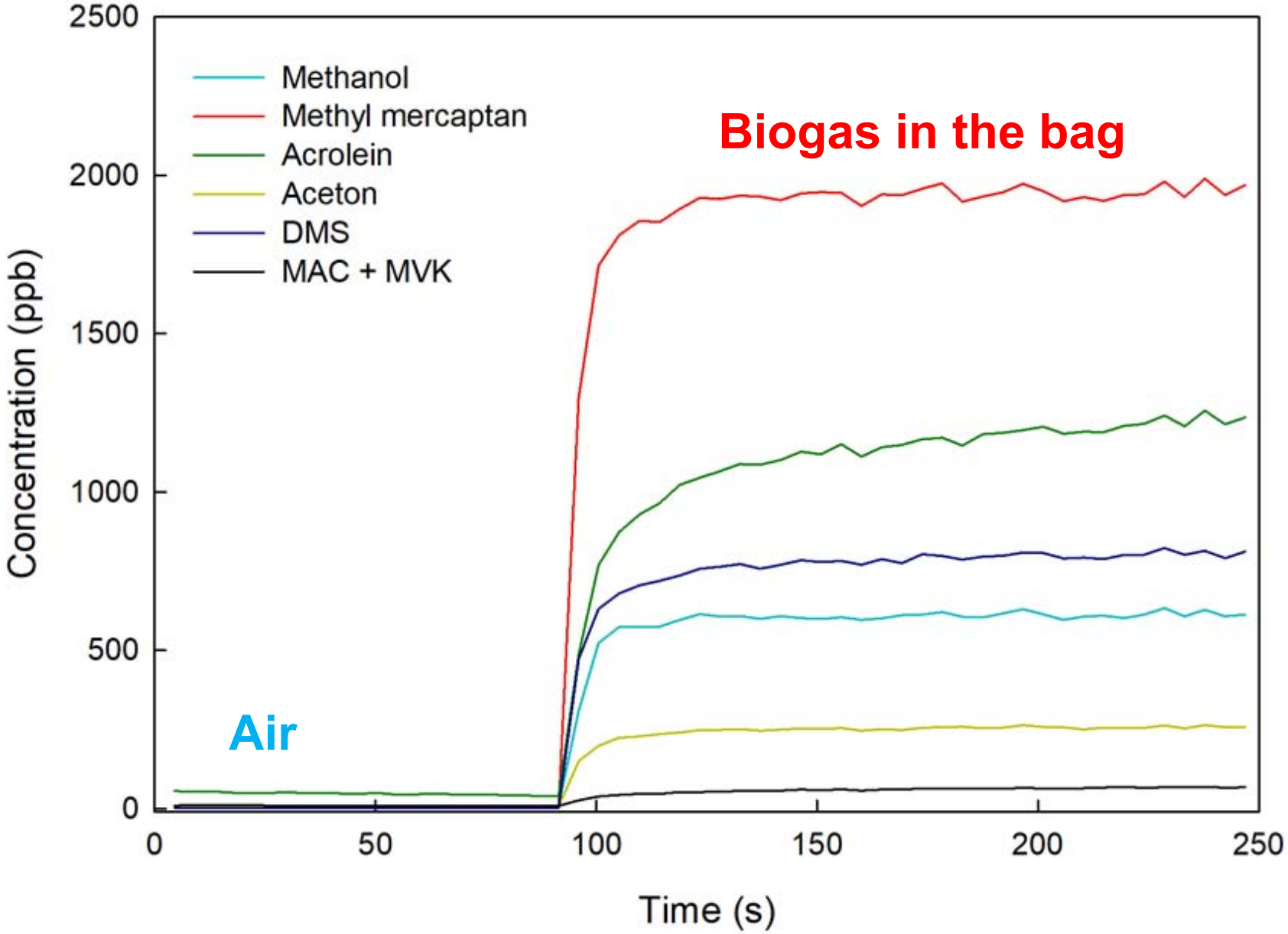
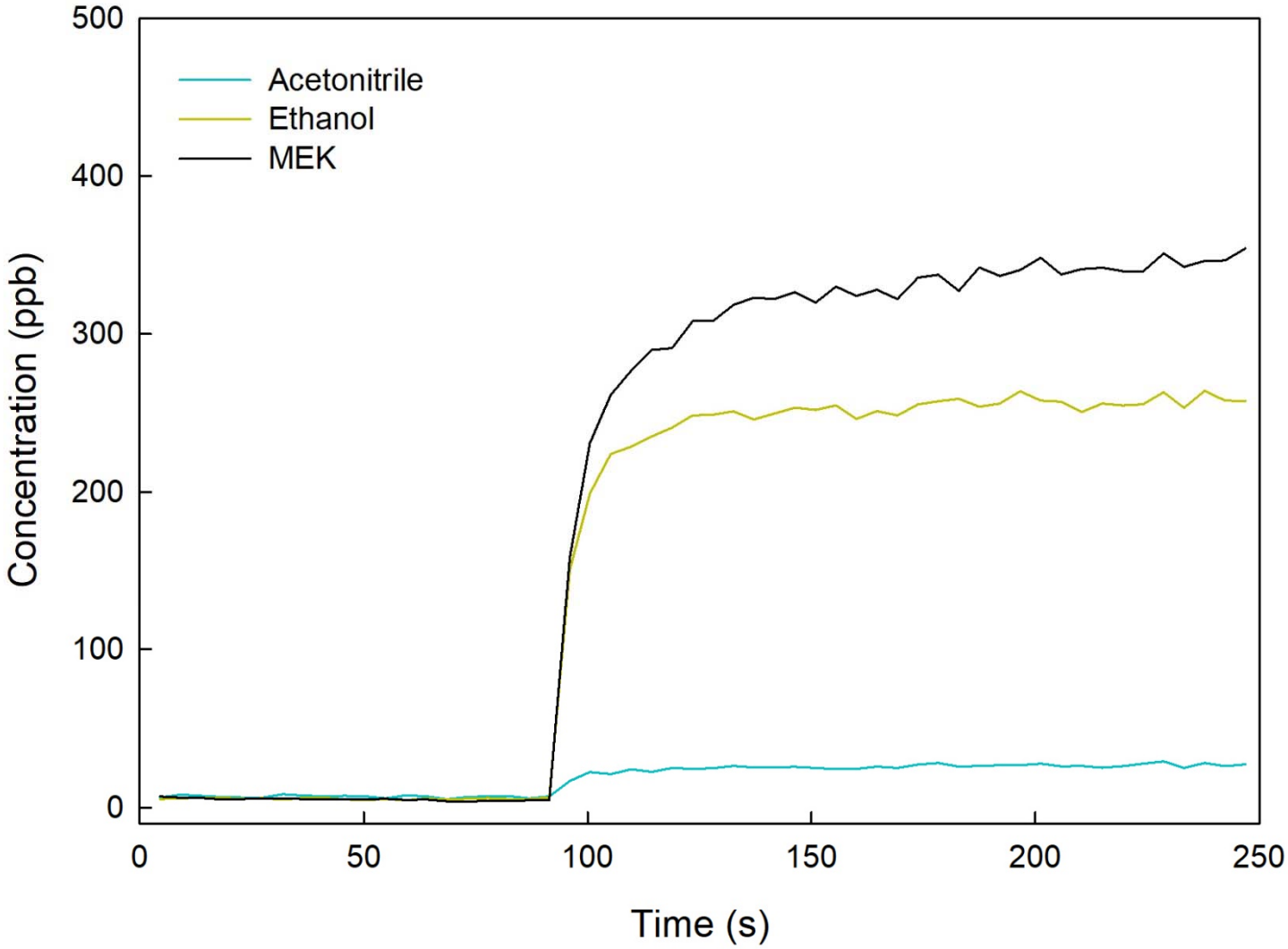


Figure (4): Spectrum measured at m/z=47. Signal intensity in arbitrary units (au). Three different peaks can be separated.

In the project, it was not possible to connect directly the instrument to the outlet of the C1 bioreactor, because the production rate of biogas (0.5 L/day) was small when compared to the inlet flow of the instrument (100 mL/min).

So 10 mL of sample were transferred in a 750 mL bag that was filled with N_2 to be analyzed by PTR-MS-TOF. Those used By Quintron for breath analysis resulted the most suitable ones for our purposes as it conserved well both permanent gases and VOCs for a few days.


Methanol
Methyl mercaptan
Acrolein
Aceton
DMS
MAC + MVK
Biogas in the bag
Air
Concentration (ppb)
Time (s)
0
500
1000
1500
2000
2500
0
50
100
150
200
250


Acetonitrile
Ethanol
MEK
Concentration (ppb)
Time (s)
0
100
200
300
400
500
0
50
100
150
200
250

VOC composition of the biogas in ppmv (Different from that shown in the Previous Figures)	
Ethanol	68.35
Methylmercaptan	51.00
Acrolein	34.00
Dimethyl Sulfide	26.30
1- Butanol	15.88
MIBK	9.70
Methylbutanols	9.34
2-Butanone (MEK)	3.88
Acetone	2.60
Acetaldehyde	2.50
1-Propanol	2.03
2-Pentanol	1.87
Mercaptoacetone	1.60
Isobutanol	1.59
Hexanol	1.20
Dimethyl Trisulfide	1.10
2-Butanol	0.79
MVK+MAC	0.66
Ethyl acetate	0.42
Dimethyl Disulfide	0.30
Carbon Disulfide	0.26
Isopropyl Alcohol	0.20
Furane	0.20
Butyl acetate	0.14
Formaldehyde	0.14
Ethyl butanoate	0.12
Methanol	0.08
Acetonitrile	0.03
TOTAL (ppm)	236.28

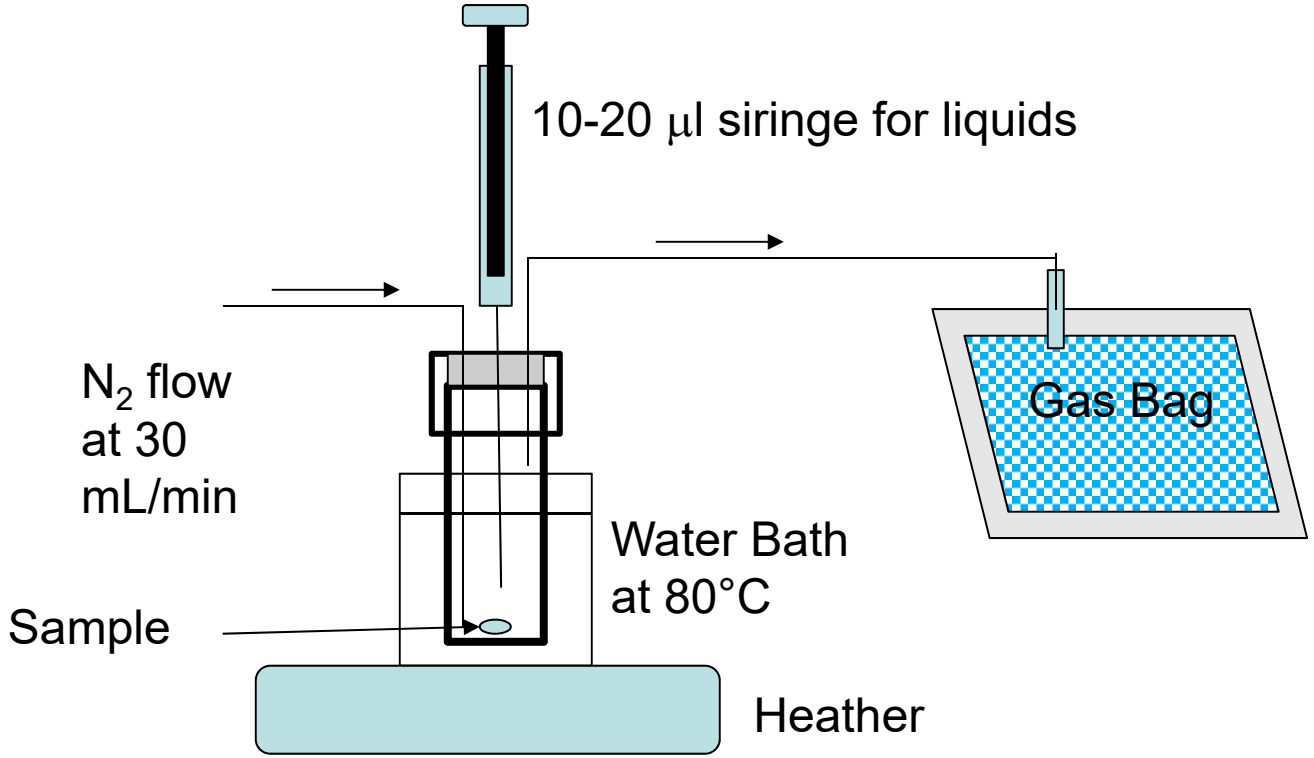
The biogas showed a quite large variability in the major components. ethanol, acrolein, methyl mercaptan and other sulfur compounds (DMS, DMDS, CS2), but the latter were always the dominant ones in the organic emission.

In particular, methyl mercaptan was usually the most abundant sulfur component, because it escapes easily from the liquid solutions, being a permanent gas at room temperature. This high levels are consistent with the fact that sulfur accounts for ca. 1% of the whole solid sample put in the C1 bioreactor.

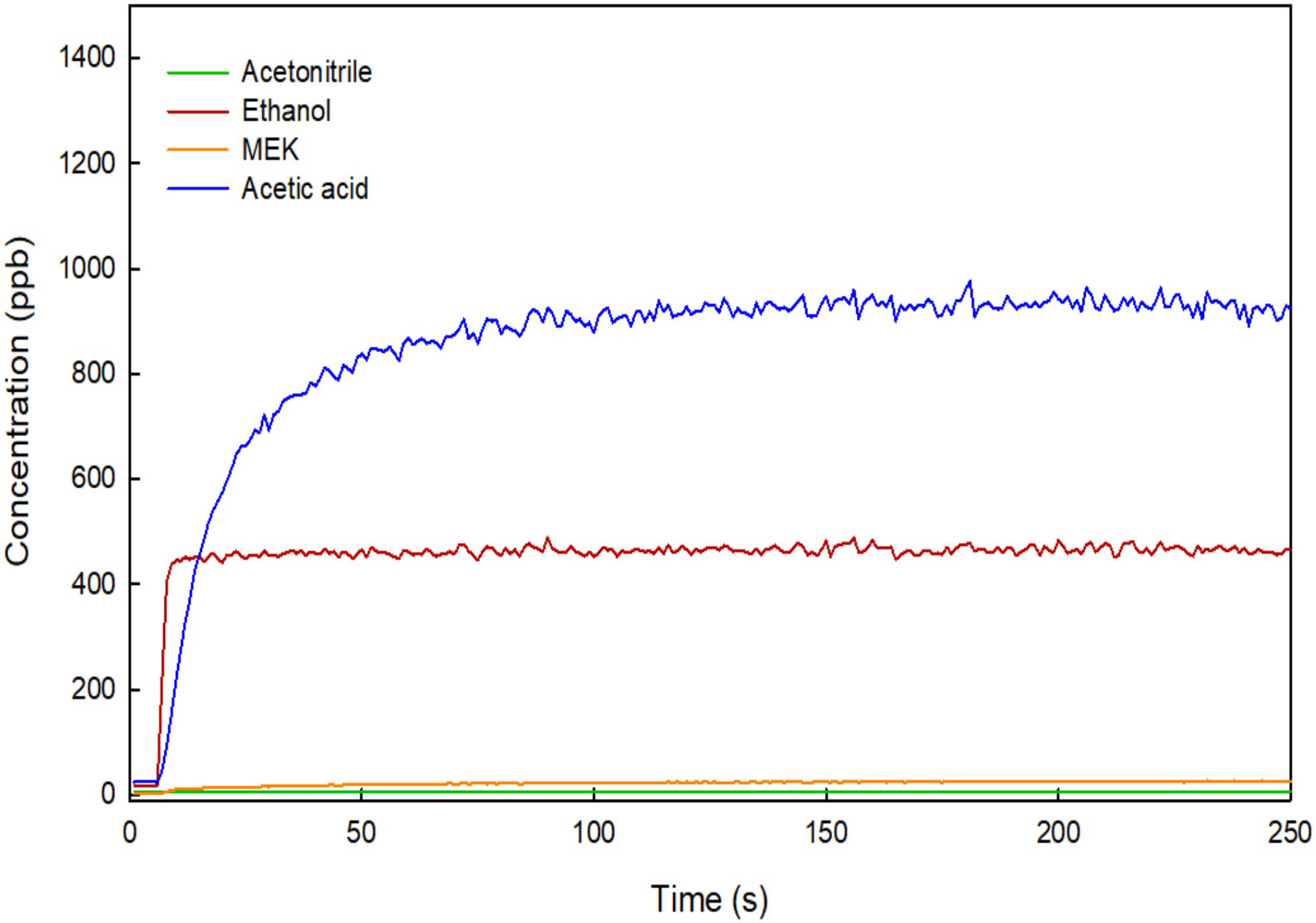
We found that also PTR-MS with quadrupole detection provides a consistent view of the VOC presengt in the biogas.

PTR-MS was also used in the analyses of liquids to complement /confirm the results obtained by ^{1}H NMR, GC-FID and GC.MS.

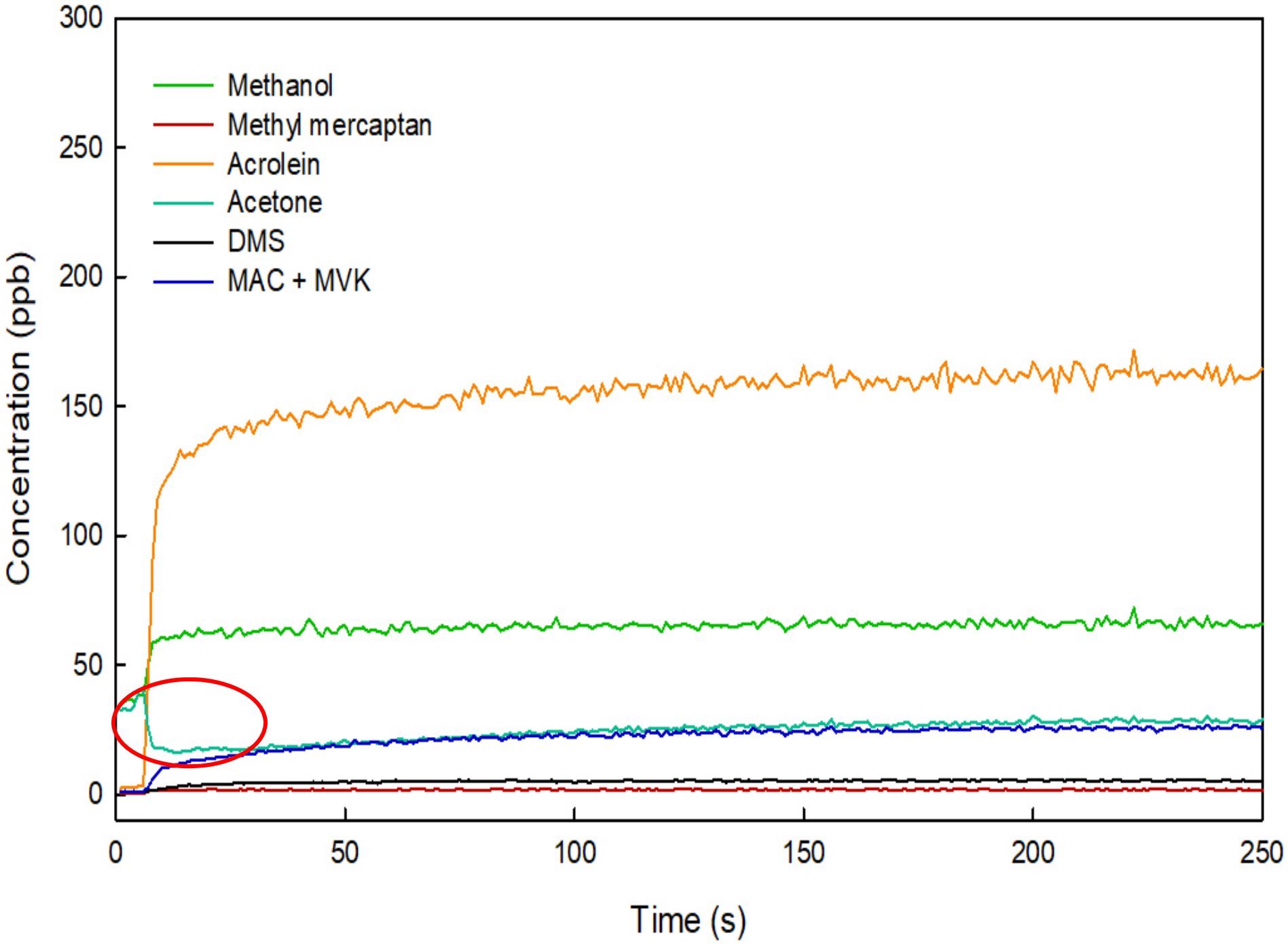
The sample collection was made by exploiting the principles of head-space technique. The procedure is illustrated in the Figure.



MEC F1


Acetonitrile
Ethanol
MEK
Acetic acid
Concentration (ppb)
0
200
400
600
800
1000
1200
1400
0
50
100
150
200
250
Time (s)

MEC F1


Methanol
Methyl mercaptan
Acrolein
Acetone
DMS
MAC + MVK
Concentration (ppb)
Time (s)
0
50
100
150
200
250
300

CONCLUSIONS

Based on the results obtained in the Project, we think that the ^{1}H NMR, ^{13}C MAS NMR and PTR-MS either TOF or Q, can be of great help in getting a better view of the composition of solids, liquid and gases produced by biological and chemical processes.

As far as PTR-MS is concerned, it can be used in spacecrafts to control the VOC content in the atmosphere, and in the various gas generation units. By considering that vacuum can be obtained from the outside space, and the water consumption is ca. 2.5 L per year, the instrument can be adapted for this specific aim.

PTR-MS can be also used for a continuous control of industrial bioreactors providing that sufficient gas production occurs from them. A dilution unit can be designed in order to introduce the amount of sample (ca. 1-100 ppbv) that can be safely detected by the instrument.

THANK YOU FOR YOUR ATTENTION!!!!!!!!!!!!!!!