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 $^1\text{H}$ NMR, $^{13}\text{C}$ 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 CO$_2$ and H$_2$) 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 CO₂ and H₂. The technical description of the FDU units and the performances obtained with them will be provided in the presentation by Amanda Luther et al.
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 consuming 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 ($B_0$).
$^{13}$C MAS NMR spectra of solids

- C=O
- Lignin
- Hemicellulose
- Cellulose

Cellulose:
- C2, C3, C5
- Amino acids
- C4a
- C6c
- C6a

Lignin:
- $\text{OCH}_3$
- a=amorphous
- c=crystal

Wood:
- Toilet Paper

Melissa Waste Sample
Batch waste C1W1

Batch waste C1W3
C1 S1 Sludge

C1 S3 Sludge
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 successful, 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 acquisition.
1H NMR SPECTRA OF THE FEEDING SOLUTIONS USED IN THE C1 REACTOR

C1 SW3

Aromatic Region

C1 SW2

C1 SW3
Expanded view of the Aromatic Region. Intensity x 128
FILTRATE SOLUTIONS FROM THE C1 REACTOR

Polyphenols and Phenols

Acetic Acid

C4 Acids
FILTRATE+PERMEATE SOLUTIONS FROM THE C1 REACTOR

Polyphenols and Phenols

Acetic Acid

C4 Acids
LIQUID MEC EFFLUENTS

MEC INFLUENT
( PERMEATE SOLUTION FROM THE C1 BIOREACTOR)

ANODE  Ion separator  CATHODE

CO₂, H₂, (CH₄) Gas production

Potentiostatically fixed anode potential
(+0.2 V vs SHE)

H₂ gas production

Influent (C1 filtrate)

Recirculate

Effluent

CH₄ + 2H₂O → CO₂ + 2H⁺ + 4e⁻

4H⁺ + 8e⁻ → 2H₂ (per liter filtrate fed to anode)

H₂O

Liquid MEC Effluents
SCWO EFFLUENT

T5 4*

4-Acetic Acid
5-Acetone
6-Metanol
8-Formic acid

T2 4

Ref
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 degradation in the C1 and FDU units.

By knowing the compounds present in the various solutions, a proper selection can be made to see what else 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 VOCs IN GAS AND LIQUID SOLUTIONS

PTR-MS exploits the ion-molecular reaction between $\text{H}_3\text{O}^+$ and a chemical species $\text{R}$ to generate ions in the MS source:

$$\text{H}_3\text{O}^+ + \text{R} \rightarrow \text{RH}^+ + \text{H}_2\text{O}$$

The reaction occurs in the MS source, only if the proton affinity (PA) of $\text{R}$ 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 $\text{H}_2\text{S}$ 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)

- **Complete & Fast** detection (whole mass spectra within 1 sec)
- **Accurate** analysis (high mass resolution, \( m/\Delta m \sim 4000 \))
  * exact mass weight
  * differentiation of isobaric species

**Primary Ions**

- \((\text{H}_2\text{O})_2\text{H}^+\)
- \(\text{H}_3\text{O}^+\)
- \((\text{H}_2\text{O})_3\text{H}^+\)

**Interesting Ions (Volatile Organic Compounds)**
In the following, the performance of the HR PTR-TOFMS is demonstrated by separation of isobars. With an example protonated ions of glyoxal (C$_{2}$H$_{2}$O$_{2}$.H$^+$) and acetone (C$_{3}$H$_{6}$O.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$_{2}$H$_{3}$O$^+$ 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\textsubscript{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.
Biogas in the bag
### VOC composition of the biogas in ppmv (Different from that shown in the Previous Figures)

<table>
<thead>
<tr>
<th>Compound</th>
<th>ppmv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>68.35</td>
</tr>
<tr>
<td>Methylmercaptan</td>
<td>51.00</td>
</tr>
<tr>
<td>Acrolein</td>
<td>34.00</td>
</tr>
<tr>
<td>Dimethyl Sulfide</td>
<td>26.30</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>15.88</td>
</tr>
<tr>
<td>MIBK</td>
<td>9.70</td>
</tr>
<tr>
<td>Methylbutanols</td>
<td>9.34</td>
</tr>
<tr>
<td>2-Butanone (MEK)</td>
<td>3.88</td>
</tr>
<tr>
<td>Acetone</td>
<td>2.60</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>2.50</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>2.03</td>
</tr>
<tr>
<td>2-Pentanol</td>
<td>1.87</td>
</tr>
<tr>
<td>Mercaptoacetone</td>
<td>1.60</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>1.59</td>
</tr>
<tr>
<td>Hexanol</td>
<td>1.20</td>
</tr>
<tr>
<td>Dimethyl Trisulfide</td>
<td>1.10</td>
</tr>
<tr>
<td>2-Butanol</td>
<td>0.79</td>
</tr>
<tr>
<td>MVK+MAC</td>
<td>0.66</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.42</td>
</tr>
<tr>
<td>Dimethyl Disulfide</td>
<td>0.30</td>
</tr>
<tr>
<td>Carbon Disulfide</td>
<td>0.26</td>
</tr>
<tr>
<td>Isopropyl Alcohol</td>
<td>0.20</td>
</tr>
<tr>
<td>Furane</td>
<td>0.20</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>0.14</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>0.14</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>0.12</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.08</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>TOTAL (ppm)</strong></td>
<td>236.28</td>
</tr>
</tbody>
</table>
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 present 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.
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!!!!!!!!!!!!!!!!!!!!!