**INTRODUCTION**

The second generation of bio refinery uses biomass with lower contents of C6 glucose and higher contents of C5 sugars. Besides ethanol generation its goal is the full usage of biomass by valorizing by products. Fermentation of C5 sugars with microorganisms result in mash that could be used for further applications. Polymer based Eurokat columns were tested for their ability to separate fermentation mash and among them the Eurokat Ca column had the best separation profile. Analysis of the mash revealed high contents of xylitol. Purification of highly pure xylitol was established.

**RESULTS**

The fermentation mash was analyzed on different columns (Eurokat Na, H, and Ca) to determine the optimal stationary phase. The Eurokat Ca column showed the best separation profile for xylitol Fig.1 even though it has the longest run with about 28 min compared to Eurokat Na with 18 min and Eurokat H with 12 min (not shown). A more detailed analysis of the fermentation mash identified five components: xylose, arabinose, glycerol, mannitol and xylitol Fig.1. Xylitol had the highest concentration with 80 mg/mL in the sample, followed by glycerol with 20 mg/mL. The other three components had concentrations of 7–8 mg/mL. The baseline separation of xylitol indicated promising batch purification. Overload studies with a semi-preparative Eurokat Ca column were performed. This column has a three-times higher volume (50 mL) than the analytical column (15 mL) and larger particle size (25–56 µm) enabling higher sample loading and faster flow rates with lower back pressure. The collected fraction of xylitol Fig.2 had a purity of 99 %, measured with RI Fig.3.

**CONCLUSION**

The Eurokat Ca column was found to be the best column for analysis of fermentation mash among tested Eurokat columns. The used fermentation mash has a high content of xylitol (80 mg/mL). A semi-preparative batch purification of the xylitol resulted in high recovery (95 %) of xylitol with a purity of 99 %. Upscaling of the batch process or application of SMB (simulated moving bed) chromatography would be promising for xylitol production from fermentation mash.

**ACKNOWLEDGEMENT**

This project has received funding from the European Union’s Seventh Framework Program for research, technological development and demonstration under grant agreement no FP7-KBBE-2013-7-613802
ADDITIONAL RESULTS

The fermentation mash was separated on Eurokat Na and Eurokat H columns Fig. A1. On the Eurokat Na column only three peaks were detected. The the Eurokat H column xylitol was also not baseline separated from the other substances. For the overload studies 50 µL to 1500 µL of the 1:10 dilution of the fermentation mash were separated on the Eurokat Ca column. Overlays of all the chromatograms show a shift in the early eluting phase (10–14 min) due to volume overload but less for xylitol Fig. A2.

ADDITIONAL MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Column</th>
<th>Column Dimensions</th>
<th>Particle (µm)</th>
<th>Eluent</th>
<th>Flow rate (mL/min)</th>
<th>Injection Volume (µL)</th>
<th>Temperature (°C)</th>
<th>Column Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurokat H</td>
<td>300 × 8 mm + 30 × 8 mm</td>
<td>10</td>
<td>H₂O/5 mM H₂SO₄</td>
<td>0.5</td>
<td>20</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td>Eurokat Ca, Na</td>
<td>300 × 8 mm + 30 × 8 mm</td>
<td>10</td>
<td>H₂O</td>
<td>0.6</td>
<td>20</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Eurokat Ca</td>
<td>250 × 16 mm</td>
<td>25-56</td>
<td>H₂O</td>
<td>2.5</td>
<td>10000</td>
<td>75</td>
<td>50</td>
</tr>
</tbody>
</table>

AZURA Analytical system

- **Instrument**: Pump
  - Description: AZURA P 6.1L, HPG, 10mL, SSst
  - Article No.: APH35EA

- **Instrument**: Autosampler
  - Description: 3950 analytical version
  - Article No.: A50070

- **Instrument**: Detector 1
  - Description: AZURA DAD 2.1L
  - Article No.: ADC01

- **Instrument**: Flow Cell
  - Description: High Sensitivity LightGuide 50 mm, 6 µL
  - Article No.: AMD59

- **Instrument**: Detector 2
  - Description: AZURA RID 2.1L
  - Article No.: ADD31

- **Instrument**: Thermostat
  - Description: AZURA CT 2.1
  - Article No.: A05852

- **Instrument**: Software
  - Description: OpenLAB® CDS EZChrom Edition
  - Article No.: A2600-1

AZURA Preparative system

- **Instrument**: Pump
  - Description: AZURA P 6.1L, HPG, 50 mL, SSst
  - Article No.: APH38FA

- **Instrument**: Autosampler
  - Description: 3950 preparative version
  - Article No.: A50054-1

- **Instrument**: Detector
  - Description: AZURA RID 2.1L
  - Article No.: ADD31

- **Instrument**: Thermostat
  - Description: AZURA CT 2.1
  - Article No.: A05852

- **Instrument**: Fraction collector
  - Description: Foxy R1
  - Article No.: A59100

- **Instrument**: Software
  - Description: OpenLAB® CDS EZChrom Edition
  - Article No.: A2600-1