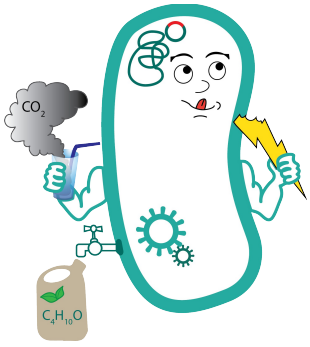
High Performance Liquid Chromatography (HPLC)

- The HPLC was used for substrate analytics. We were able to detect glucose, xylose, succinate and fumarate in the media.
- Sample preparation:
  - Centrifuge 1.5 ml of bacteria culture for 5 minutes at 14.000 rpm
  - Transfer supernatant to a fresh tube. Avoid to resuspend the cells
  - Add 2 µl of sodium azide (100g/ L) to each sample.
  - Store the samples at -20 °C untill you analyse them by HPLC
- HPLC measurement:
  - 750 µl of each sample are transferred into HPLC vials with a septum
  - The samples are determined in duplicates together with two sets standards á three concentrations
  - The concentration of the standards depends on the maximum expected carbon source concentration in the media
- Sample run:
  - It was used the *VA 300/7.7 NUCLEOGEL SUGAR 810 H* column for sample separation
  - Eluent was 3.5 mM H_2SO_4
  - The column was heated at 65 °C and the injected sample volume was 20 µl
  - Detection was achieved by conductivity measurement



- For performing enzyme assays with purified enzymes or crude cell extracts we were using following HPLC-MC system.
- HPLC: LaChromUltra (Hitachi Europe, United Kingdom)
- Analysis: microTOF-Q hybrid quadrupole / time of flight mass spectrometer (Bruker Daltonics, Bremen, Germany)
- Ionization: Electrospray ionization (ESI)
- Column: SeQuant ZICpHILLIC column (150 by 2.1 mm, Merck KGaA, Darmstadt, Germany)
- Solutions: 10 mM Ammonium bicarbonate solution (eluent A), acetonitrile (eluent B)
- Injection volume: 2 µl
- Flow rate: 150 µl min^{-1}
- Gradient [t_{min}, % of eluent B]: t_0: 80%, t_{30}: 10%, t_{35}: 10%, t_{40}: 80%, t_{60}: 80%
- Negative ionization mode
- Internal mass calibration: Formate (0.1 M), in 50% (vol/vol) isopropanol