Online IR monitoring of a fermentation process

dsk.2020

A horror story featuring blood, sweat, tears, and tremendous success and failure

Lars Houmøller
Glycom Manufacturing
lars.houmoller@dsm.com

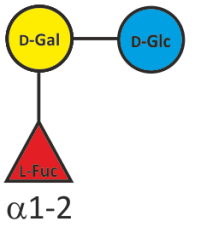
NUTRITION · HEALTH · SUSTAINABLE LIVING

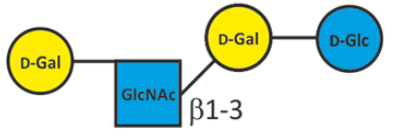
DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

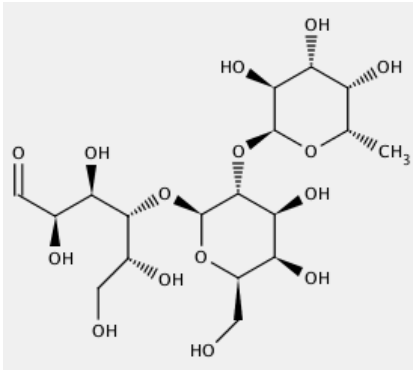
Glycom intro

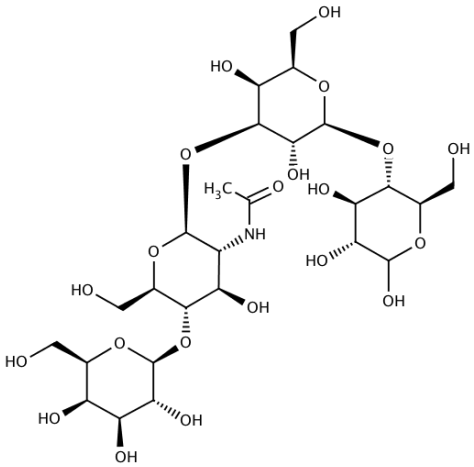
Human milk oligosaccharides (HMOs)

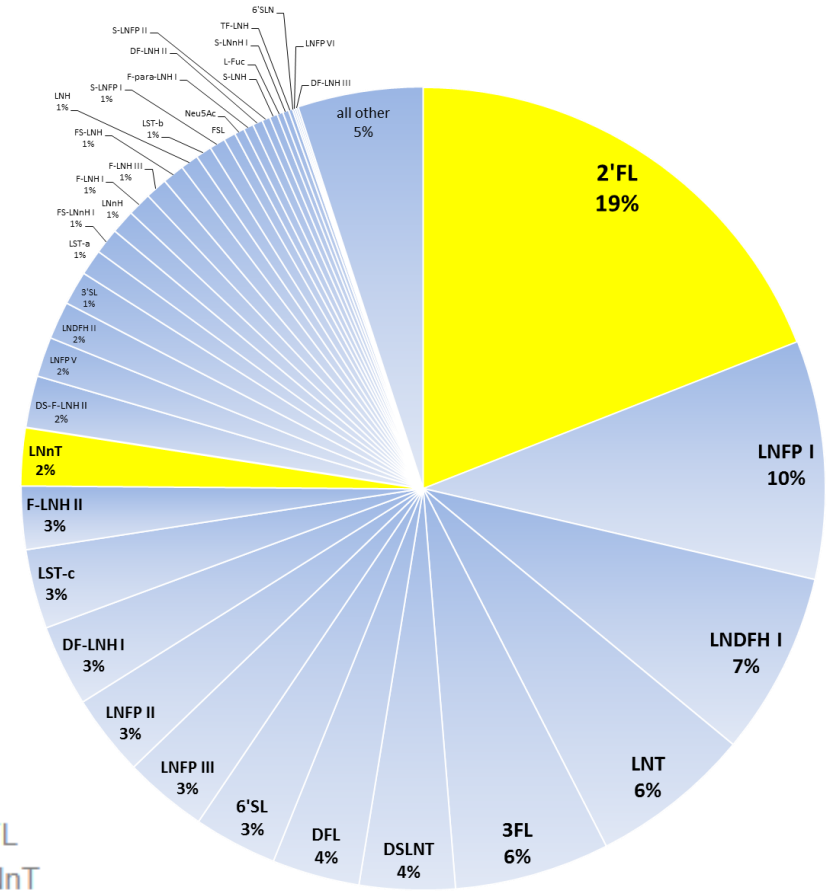
>200 HMOs identified
12 most abundant HMOs ~75% of total











Production

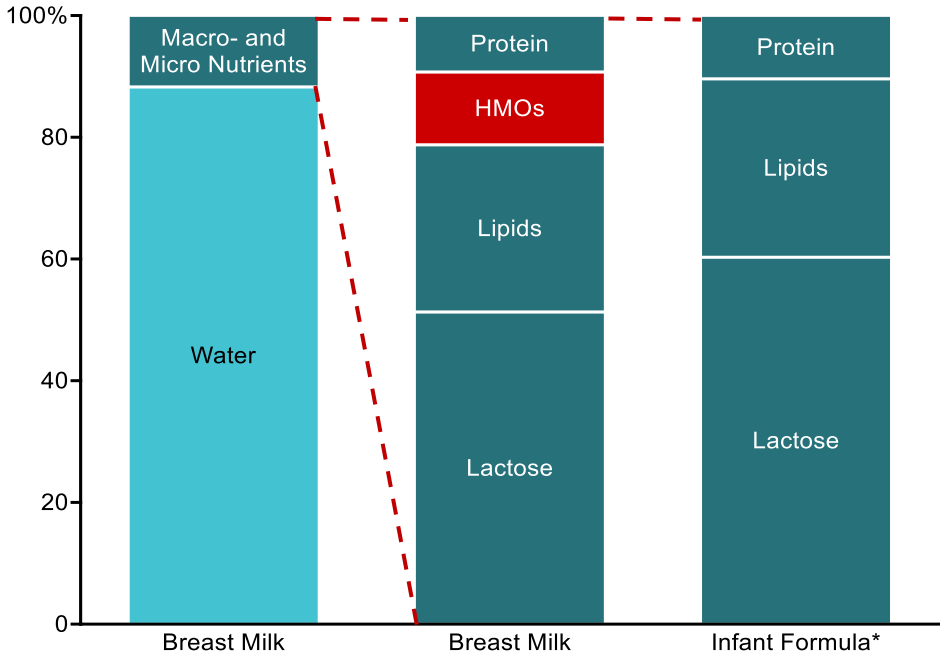
1. Aerobic fermentation by modified *E. Coli*.
2. A lot of purification steps
   → very pure HMO (white powder).

Products

GlyCare™ 2FL
GlyCare™ LNnT
GlyCare™ 2FL/DFL
Glycare™ LNT
GlyCare™ 3SL
GlyCare™ 6SL
GlyCare™ LNFP I
GlyCare™ 3FL

Use of HMOs

Composition of breast milk and infant formula:



- **HMOs have until very recently not been present in infant formula.** HMOs are oligosaccharides (sugars) which form the 3rd largest component of human milk
- Abbott and Nestlé have launched first products containing HMOs in 2018
- HMOs have major benefits for all ages in conditions involving compromised/inflamed intestinal barrier and regulation of immune system
- HMOs **improve gut health**
- HMOs **protect the infant from bacterial and viral infection**
- HMOs **support immune responses**

Products for adults:

Living with IBS?

Holigos® IBS Restore is a medical food proven to nutritionally manage IBS symptoms like abdominal pain, bloating, constipation, and diarrhea.

SHOP NOW

Occassional digestive issues?

Holigos® Maintain is a dietary supplement for proactive digestive health and support with occasional digestive issues.†

SHOP NOW

Online monitoring of fermentation

Near infrared spectroscopy

During production of lysine at VitaLys, online NIR was implemented on the outflows from two fermenters (450 m^3 each).
Advantage: no requirements for sterility (outside the fermenters).
Problems:

- Variations in flow and temperature
- Air bubbles
- Cleaning of the flow cell (path length 1 mm)

Mid-infrared spectroscopy

Probe inserted directly in the fermenter.
Advantages:

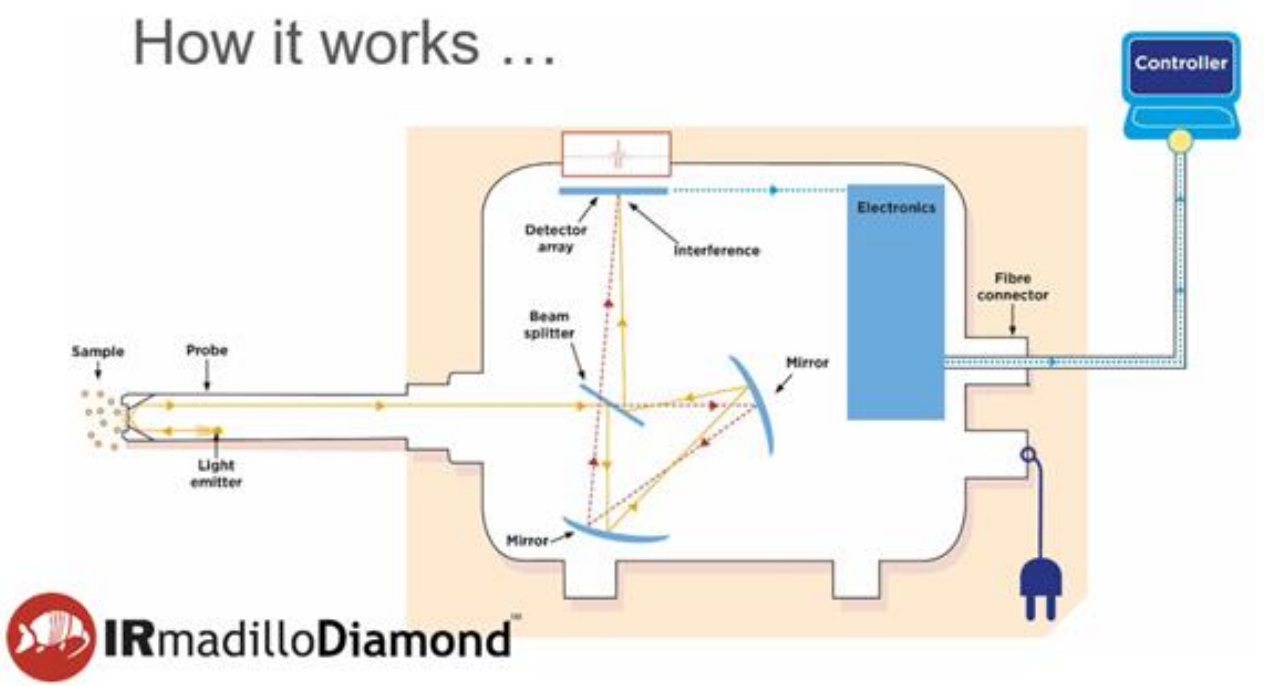
- Cleaning performed during CIP of the fermenter
- Using ATR technique, no problems with air bubbles (only a few micrometers of the liquid is probed)
- Mid-IR better for discriminating different sugars

Instrumentation

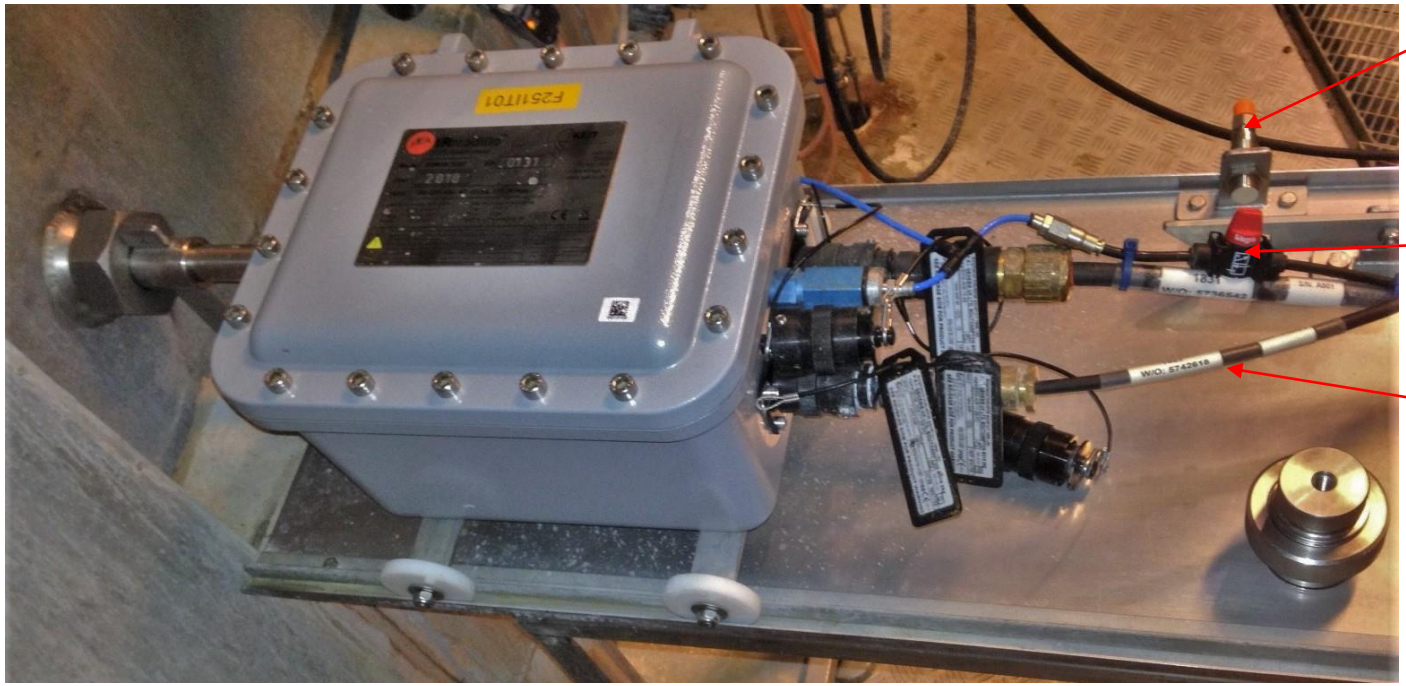
IRmadillo from Keit Spectrometers (UK)

- No design awards...
- Diamond ATR probe.
- Wavenumber range: 848-4000 cm^{-1}.
- Fourier transform instrument with no moving parts.

Pink fairy armadillo



Installation



Switch (instrument out)

Dry air (constant purge)

Optical fiber
(data transfer)

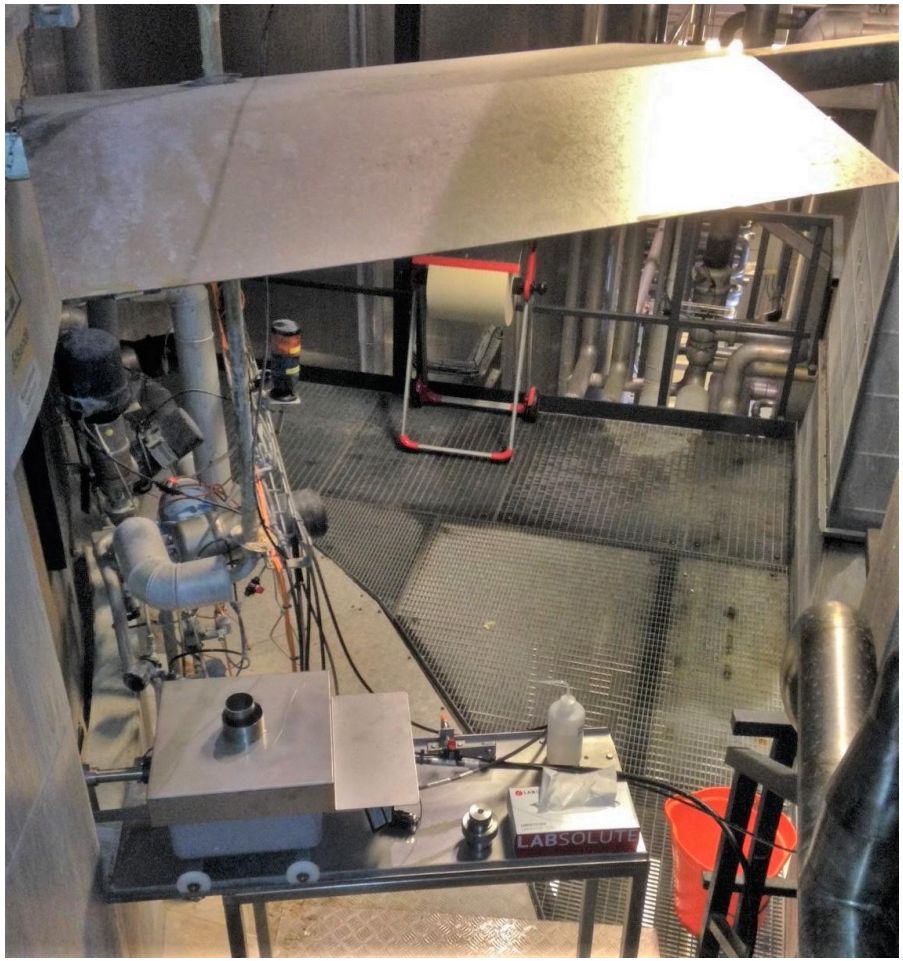
ATR probe (glass)

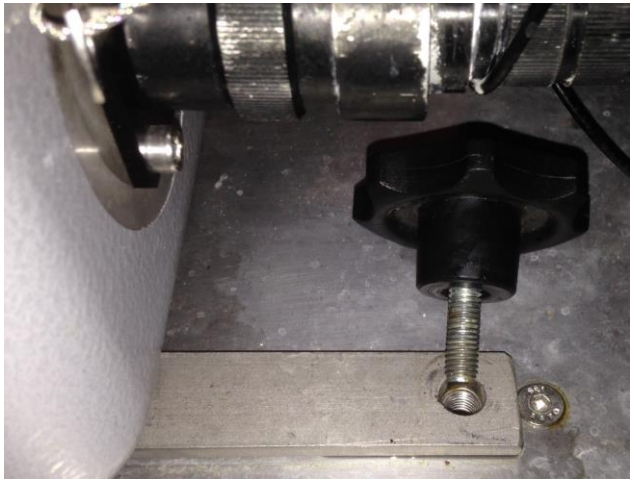
Protection cap

Plug for port

Installation

Roof and cover installed after splashing of instrument

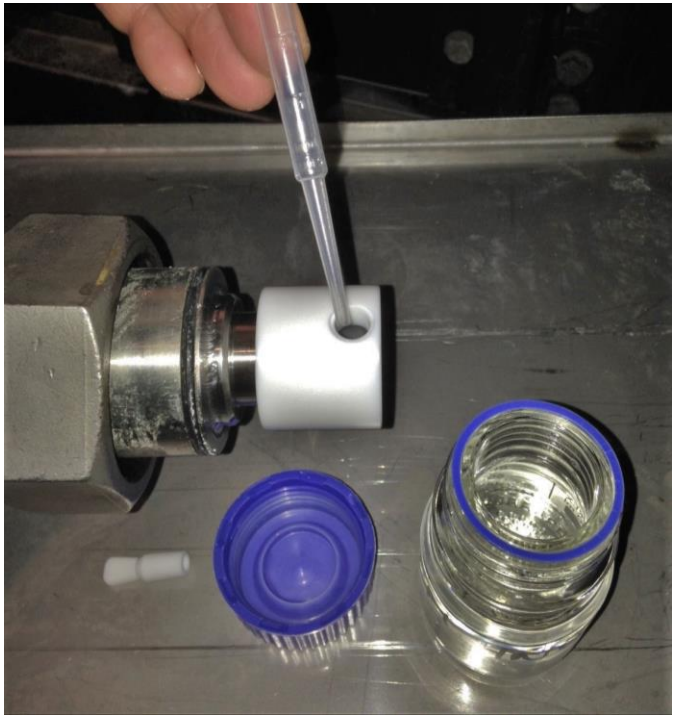




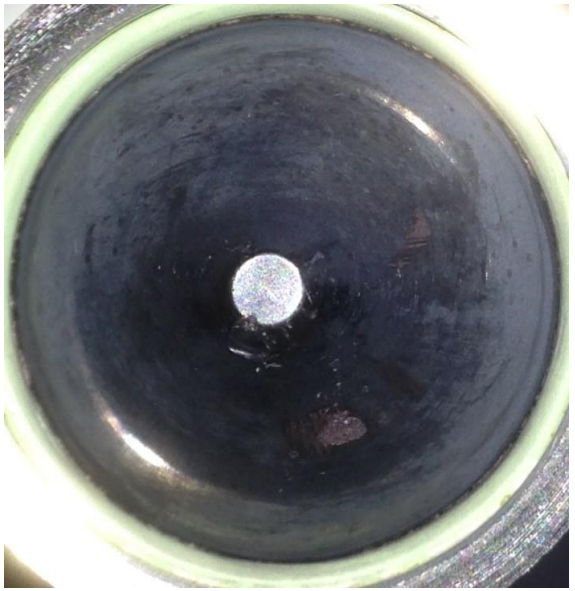
Instrument secured with screw.
Forgotten by operator ⇒
instrument pushed out by pressure
in fermenter during SIP ⇒
steaming of instrument
(bonus info: not optimal...)

Glass ATR probe

Cleaning wit nitric acid, water, and lint free paper



“Blooming” on surface (probe extracted during CIP)



Instrument returned, glass investigated by SEM etc.; no explanation

Diamond probe; 2nd instrument; pixels problem

Diamond probe

Glass ATR exchanged with diamond (less sensitive; only one bounce)

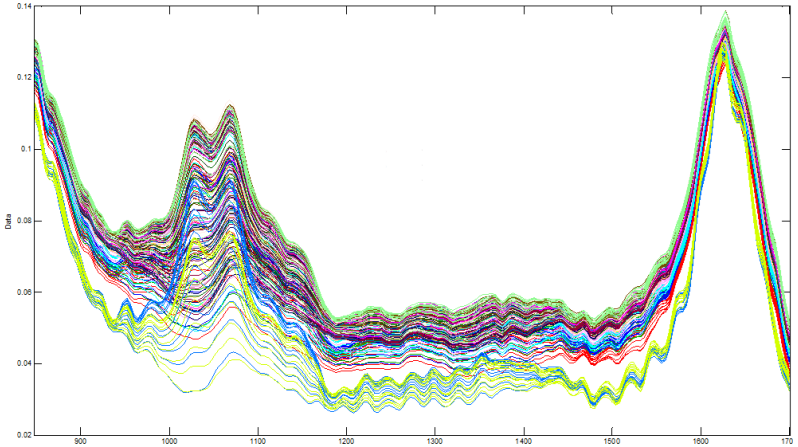
Probe not extracted during CIP

First probe installed and used during fermentation ⇒ useless spectra and results.
Found out that probe was damaged during transportation; Keit technician arrived and changed.

2nd instrument

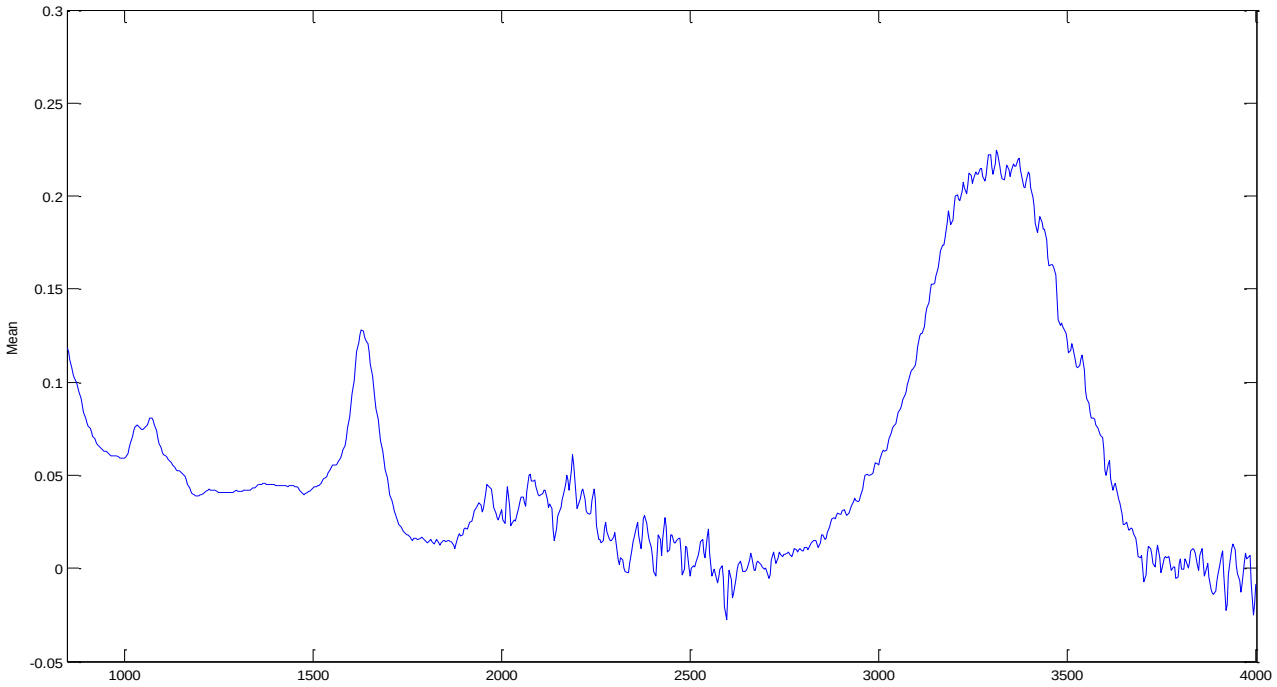
Second instrument purchased; 1 Keit salesperson and 1 technician spent 2 days analyzing standard chemicals for calibration transfer between instruments.
Mistakes during measurements ⇒ time wasted.

Pixels problem



Sudden change in spectra: “waves”:
Effect of defect pixels.
Values of non-working pixels set to mean of neighbors.

Spectra, calibrations, and predictions



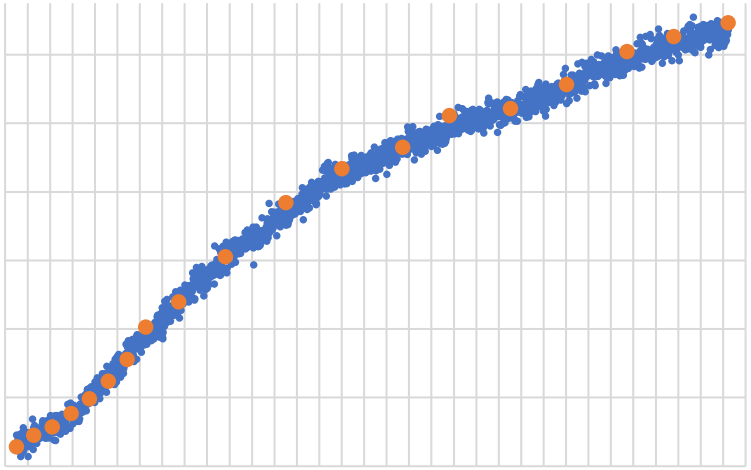
Above: mean of 2118 spectra for selected batch.

- For calibrations, 848-1700 cm^{-1} is used followed by variable selection.
- Most common pretreatment: mean centering + 1st derivative.
- Software: *Solo* + *Model_Exporter* (Eigenvector).
- Models imported in *Keit Spec* software.
- Predictions exported to automation software (*FoxPro*); shown on screens in control room and available for process engineers for monitoring and optimization.

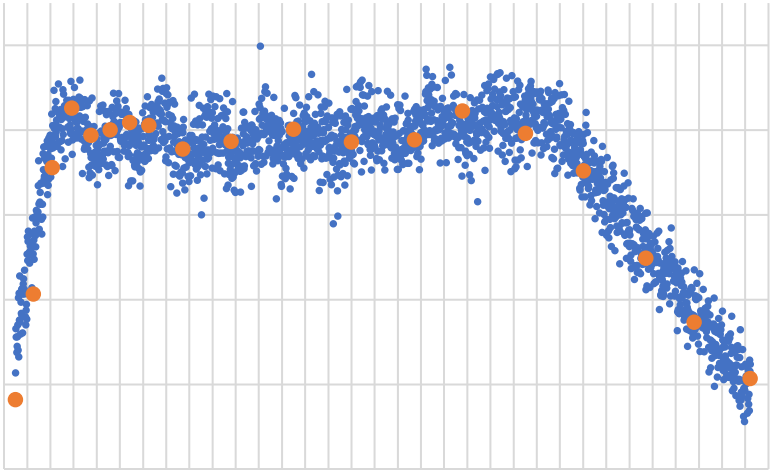
Results I

Prediction of OD and selected constituents (names and units unfortunately forgotten).
Blue dots: Predictions; orange dots: lab results.

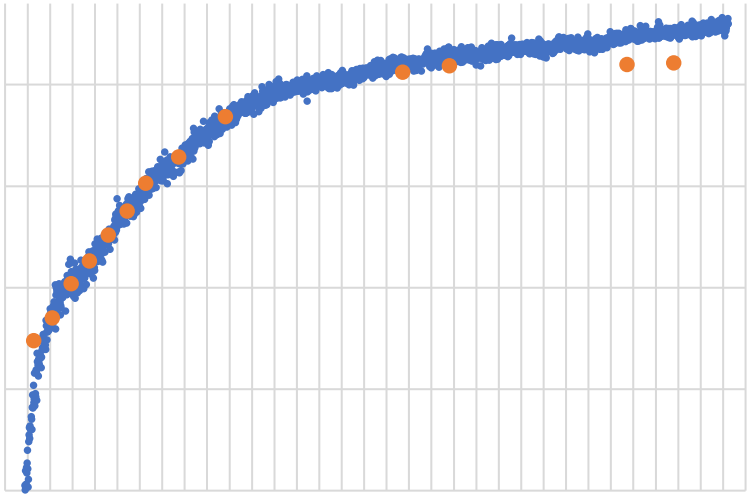
Product A, Component 1



Product A, Component 2

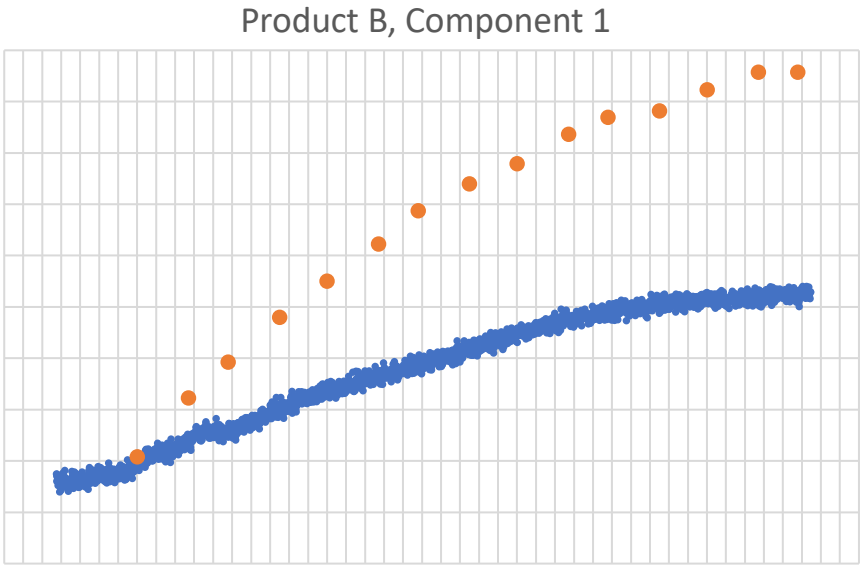


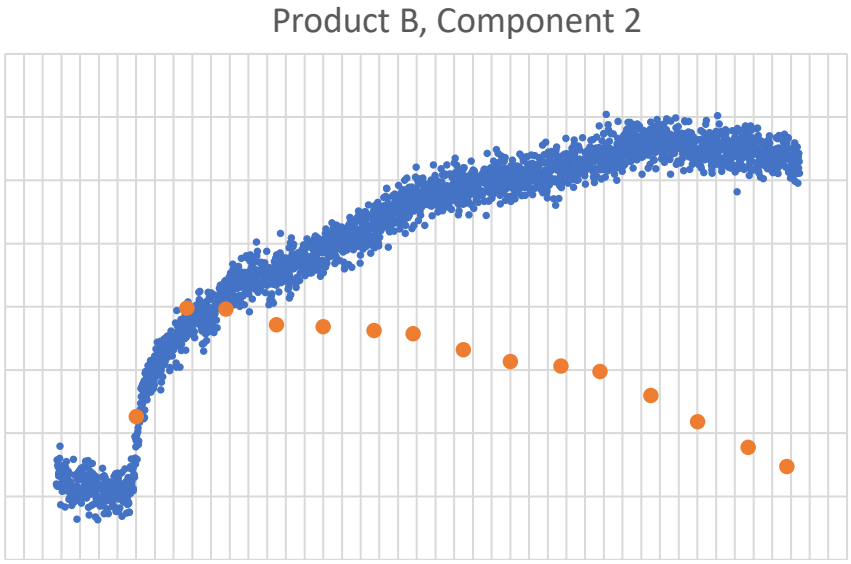
Product A, OD

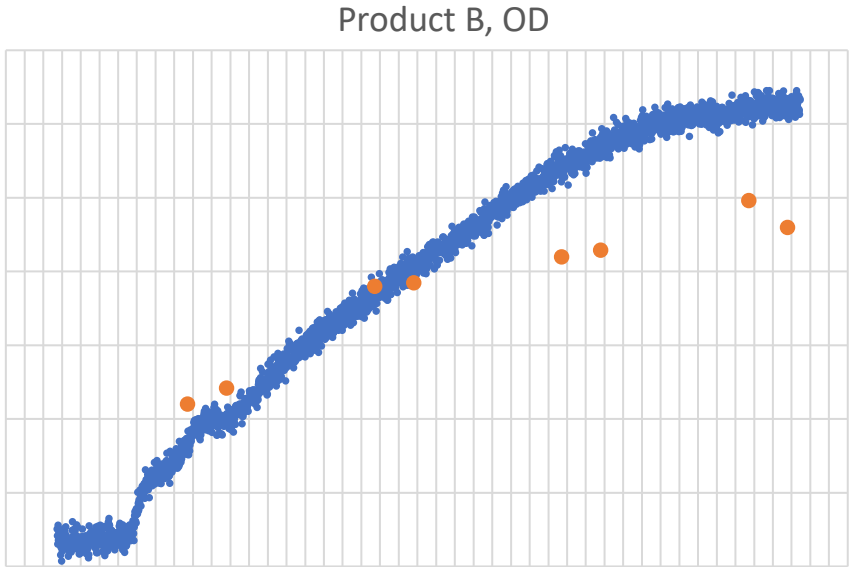


- Results shown are raw predictions (every 2 minutes based on average of 12 scans).
- To compensate for variations in predictions, running average of 5 predictions are used for process monitoring.

Results II: Test of new bacterial strain







Conclusions

- Don't use the new strain (although yield is much higher); doesn't fit existing calibrations.
- Close down R&D; apparently only aim is to annoy hardworking chemometricians.