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iConsensus WP5: Spectroscopy and chemometrics support

Bioprocess monitoring with Raman spectra Generic data driven models?

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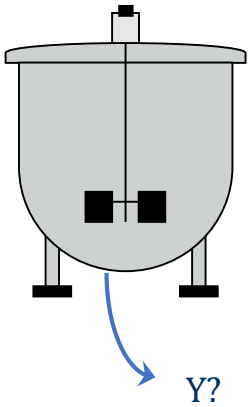
Chemometrics

(and other "…ometrics" such as biometrics, econometrics, psychometrics, …)



Problem:

System or (bio)process with **interesting properties/variables** (Y) that are **not easily accessible**.

Idea:

Use some sort of mathematical model (f) to **calculate** the **interesting part** (Y) **from** other process information that is more **easily accessible** (X).

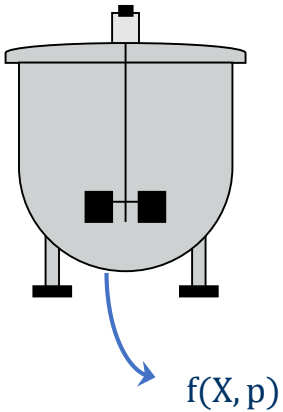
$$Y = f(X, p)$$

Easily available process information (X); wish list:

Easy and (relatively) cheap to measure
Fast, real-time capable measurement
Non-invasive/non-intrusive measurement
…

} Spectroscopy!

Chemometrics



$$Y = f(X, p)$$

What about p?:

Perform experiments; measure spectra (X) as well as difficult to obtain target information (Y)

Calibrate the model = determine p

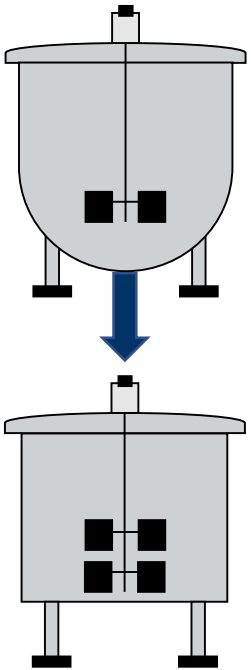
What about f? Which model to chose?:

Keep it simple! (as simple as possible, as complex as necessary to do the job)

Complex models → not necessary or not possible (lack of sufficient data)

Partial least squares regression (PLS-R) with basic pre-processing is fine in most cases!

“Generic” chemometric model?



Key challenge:

Genericity of data driven statistical models is usually very bad.

Small change in the system/(bio)process

→ chemometric models invalidated! New calibration is necessary!

High calibration effort → it might be easier to:

- Measure the variable of interest with the old offline method!
- Ignore the variable if possible!

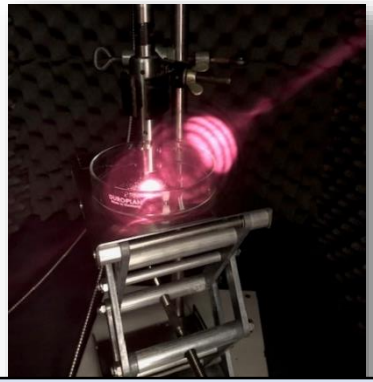
Verdict: Spectroscopy based data-driven models can be awesome, if the work properly **but** they can be very difficult to use in a lot of cases.

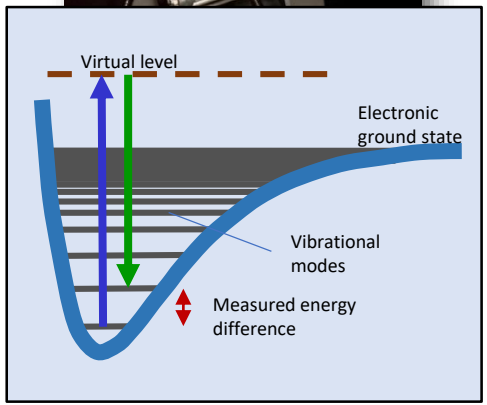
Solutions?

Trade accuracy for reliability/flexibility

- Use the right spectroscopic tool!
  - Avoid “indirect” measurements!
  - For chemical composition, Raman or TF-MIR is a good start
- Calibration dataset: Have as much variability as possible!
  - User different processes and hardware to collect data
  - Collect data from multiple sites
- Keep the pre-processing to a minimum!
  - Data alignment is necessary (probably)
  - Everything else is optional (depends on the actual data and process)
- Use simple regression model!
  - No need for fancy models here. Simple PLS-R is perfectly adequate.
  - The less parameters, the better!

Raman spectroscopy





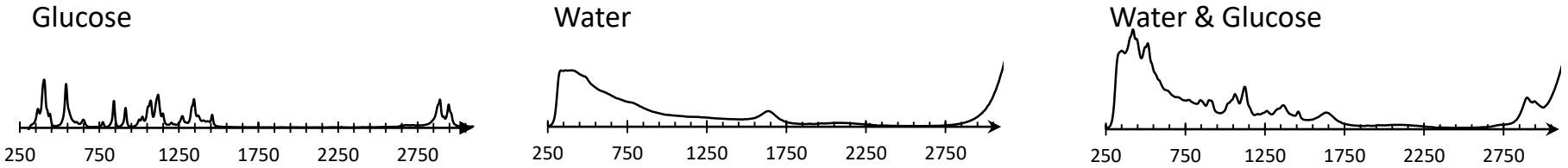
Raman scattering is caused by excitation of vibrational modes in the sample molecules

→Vibrational spectroscopy

Measured energy differences are ~ 0.05 – 0.5 eV

Comparable to MIR spectroscopy (sort of) but shifted to higher energies → water absorption of MIR is no problem in Raman spectroscopy

Raman spectroscopy



Raman effect is linear in nature! A Raman spectrum of a sample is pretty much the sum of all the spectra of the individual compounds in the sample!

→Raman spectra can be used to determine the composition of a mixture different compounds!

But there is **fluorescence!**

Very strong in biological systems, which can make the evaluation of Raman spectra very difficult.

Calibration

- Capture as much variability as possible in the calibration data set!
- Measure Raman spectra as well as target variables/compounds!
- Vary process conditions!
  - Different environmental conditions
  - Different feed strategies
  - Different cell lines
- Measure at different sites (if possible)!
- Use different spectroscopic hardware (if available)!

Calibration data

iConsensus → access to large amounts of process data from 4 companies (Bayer AG, GSK, Sanofi, Rentschler Biopharma)

We picked a small subset (most recent):

- 41 different cultivation batches in total.
- 1699 data points (Raman spectrum & off-line measurement).
- Raman spectra acquired with different spectrometers (Kaiser RXN2, Resolution ProCellics Raman analyzer).

Validation data

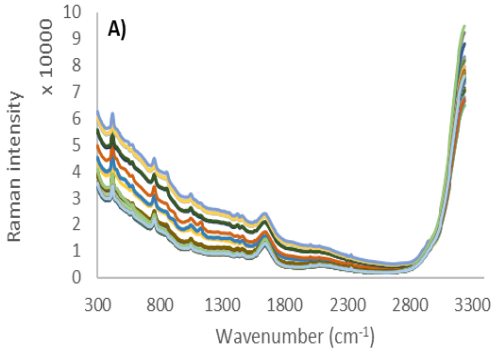
1. Validation data that is **similar** to calibration/training data:

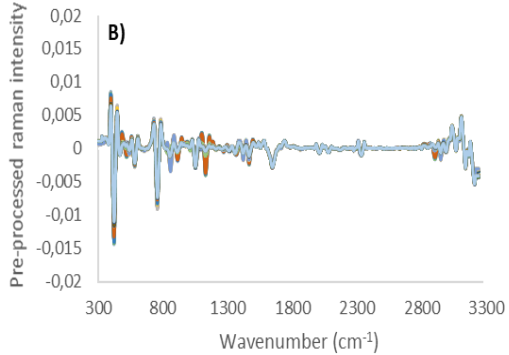
 > 10.000 data points from iConsensus.

2. Validation data that is **different** to calibration/training data:

- 240 data points form dilution series of major compounds in water and FMX-8 medium
- 550 data points from test cultivations at Royal Institute of Technology in Stockholm, group of Veronique Chotteau

Preprocessing of spectra





Keep the pre-processing to a minimum!

1. Plausibility checking/outlier detection and data alignment:
   - Interpolation
   - Intensity correction

2. Baseline/fluorescence removal:
   - High pass filter
   - First derivative Savitzky-Golay filter

3. Normalization and removal of any remaining linear offset:
   - SNV normalization

Chemometric model

$$Y = L_0 + L_1x_1 + L_2x_2 + L_3x_3 + \cdots + L_nx_n$$

$$Y = f(X, p)$$

Spectra → X:
Pre-processed Raman spectra, 1001 channels

Target variables → Y:
glucose, lactate, glutamine and glutamate

Model f:

Type of regression model	Validation data	
	Similar to calibration set	Independent data set
Neural networks (MLP, CNN,…)	Very good ✓	Very bad ✗
Gaussian process regression	Good ✓	Bad ✗
Linear regression (MLR, PCR, PLSR,…)	Reasonable ✓	Reasonable ✓

"accuracy" decreases

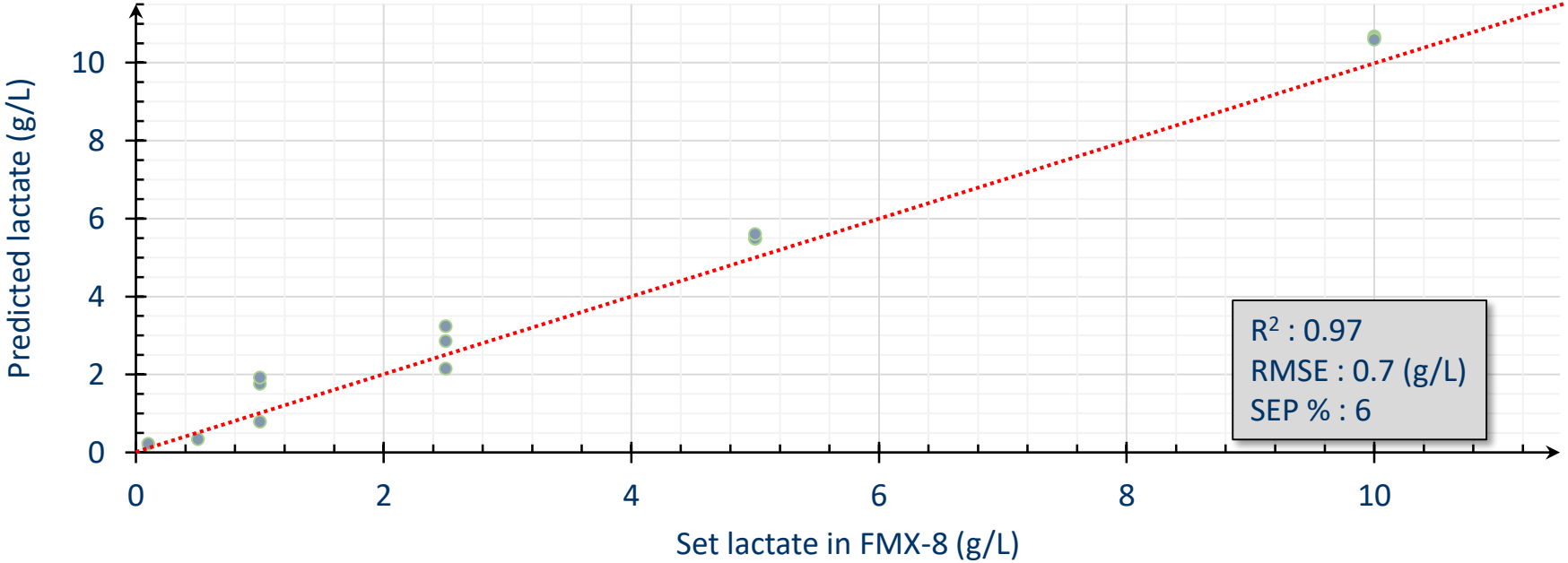
"flexibility" increases

✓ = "good enough"

Results

Independent dataset 1:

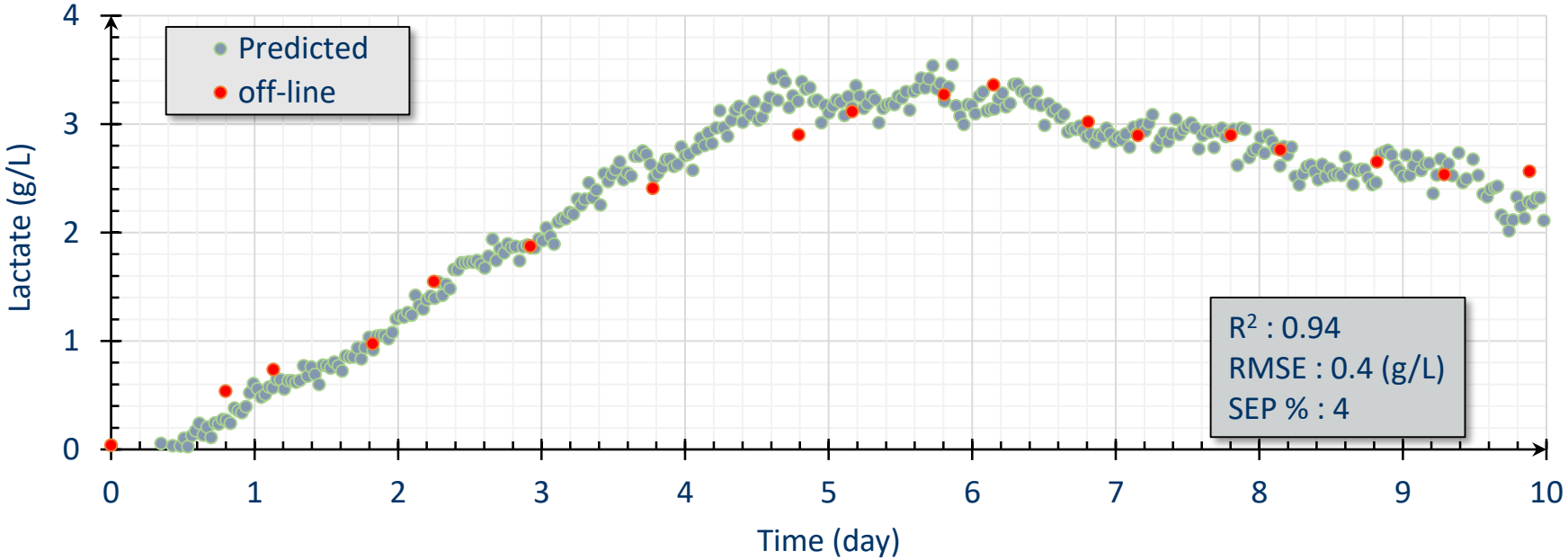
- From University of Hohenheim
- Spectrometer: Tec5 Raman 785 with modified InPhotonics RPS785/FF12 probe
- Dilution series of various compounds in water and FMX-8 mod medium



Results

Independent dataset 2:

- From Royal Institute of Technology in Stockholm
- Spectrometer: Kaiser RXN2 Raman analyzer
- Fed batch cultivation of CHO TurboCell (TM)



Conclusion

If we are willing and able to trade accuracy for reliability/flexibility:

Generic and flexible data driven **models are possible**!

But:
Higher effort for calibration required!
Generic models are often less accurate (but can still be “good enough”)!

Discussion / Questions?

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