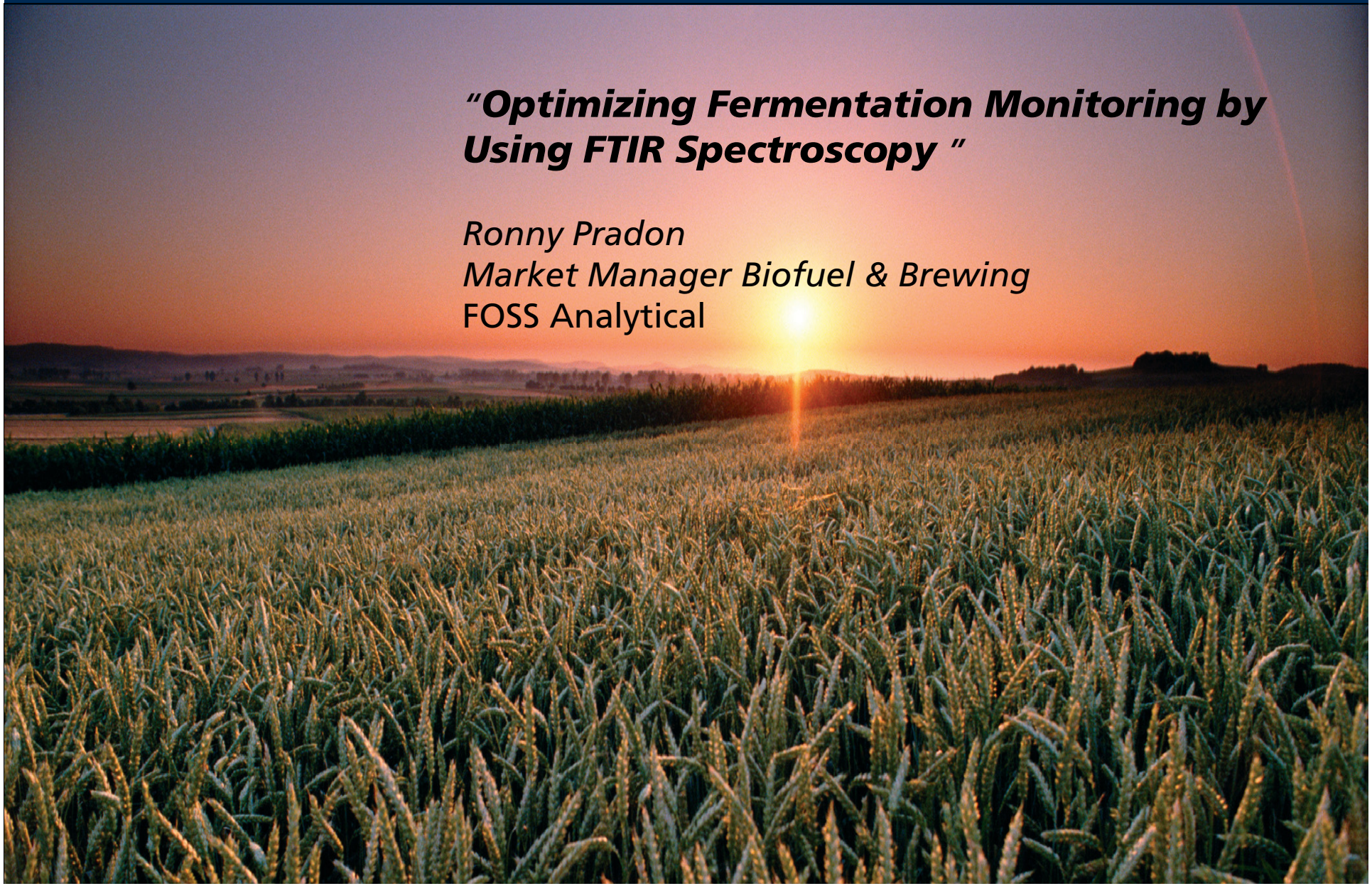
FOSS – October 14, 2008

FOSS



"Optimizing Fermentation Monitoring by Using FTIR Spectroscopy "

Ronny Pradon
Market Manager Biofuel & Brewing
FOSS Analytical

Agenda

FOSS

Introduction to FOSS

Current use of spectroscopy throughout ethanol production

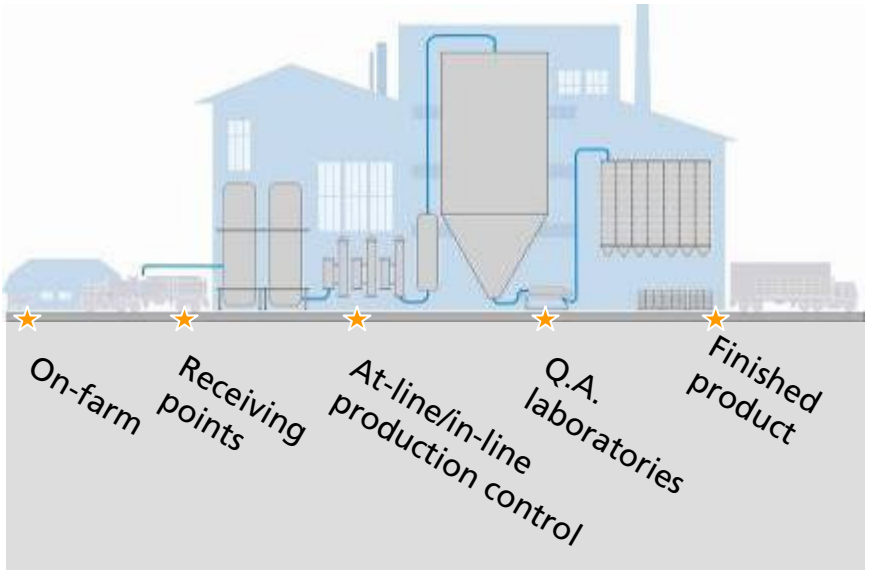
FTIR for accurate fermentation monitoring

Summary

Improving quality and profitability from raw material to finished product

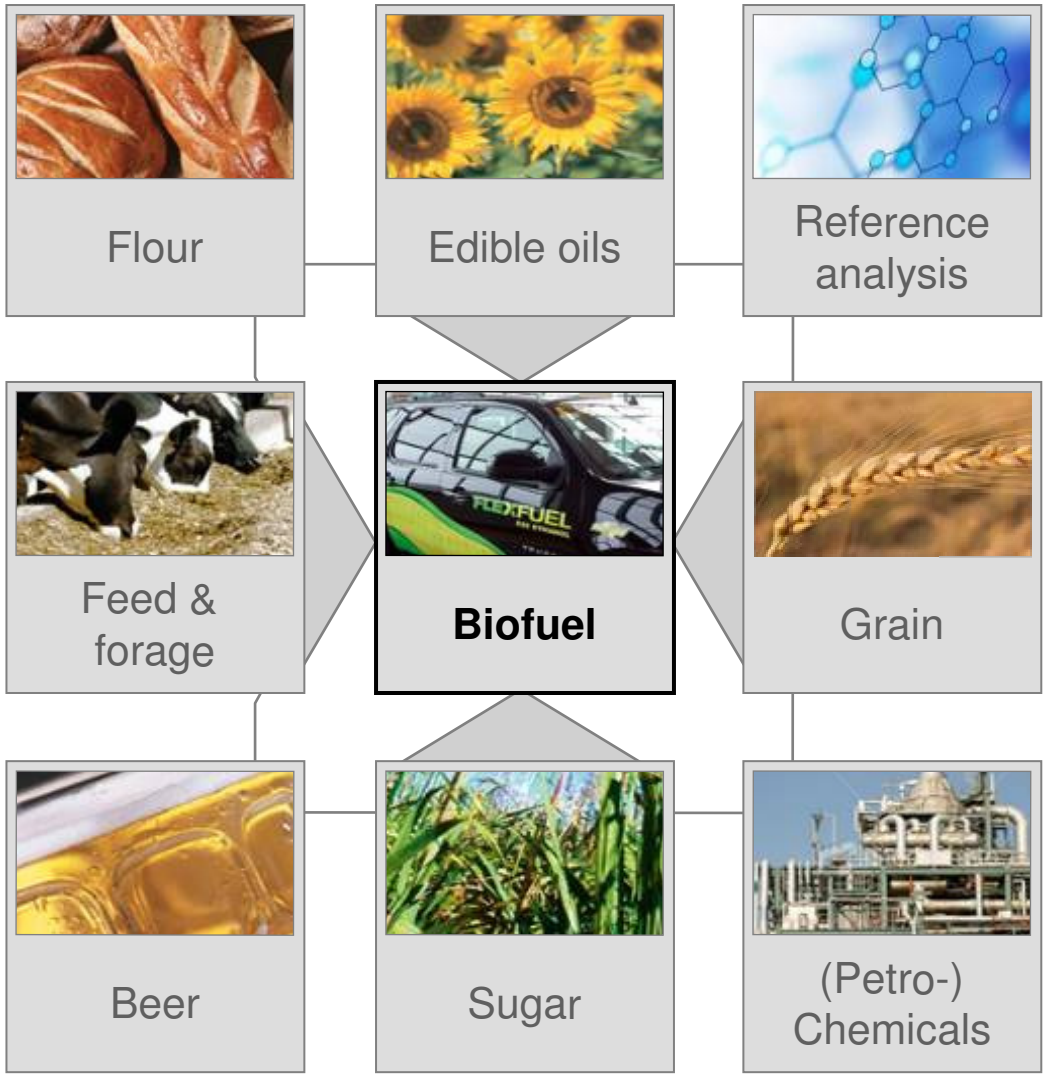
FOSS provides rapid, reliable and *dedicated analytical solutions* for routine control of quality and processing of agricultural, food, pharmaceutical and chemical products

Improving quality and profitability from raw material to finished product



Concentrating all relevant experience and expertise in a dedicated business segment.

FOSS



A leading player in the global NIR and FTIR market

FOSS

- Innovator in utilizing spectroscopy for more than 35 years
- Strong software platform for routine operation and calibration development
- Research partnerships with leading international universities and technology groups
- Combines chemical analysis, precision mechanics, electronics, software, optics and advanced chemometrics

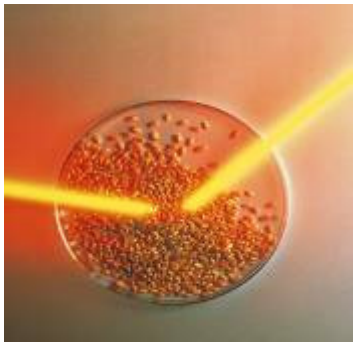
Highlights :

- **100+ patents relating to NIR / FTIR technology**
- **20+ world first introductions**
- **45 state-of-the-art solutions**
- **First to introduce advanced analysis outside of safe laboratory environment**

How does it work

FOSS

1. Light is reflected onto or through a sample (reflectance / transmission).
2. The light will be modified (partially absorbed) by the composition of the sample.
3. The remaining light is picked up by a detector giving us a spectral fingerprint of the sample.
4. Combining this spectral information with reference values using mathematics (chemometric) gives us an algorithm (calibration model).
5. Applying this calibration model on an unknown sample allows us to calculate its composition.
6. The performance of the results is a combination of good spectrometers and powerful chemometric tools plus accurate reference values when building the calibration models.



Agenda

FOSS

Introduction to FOSS

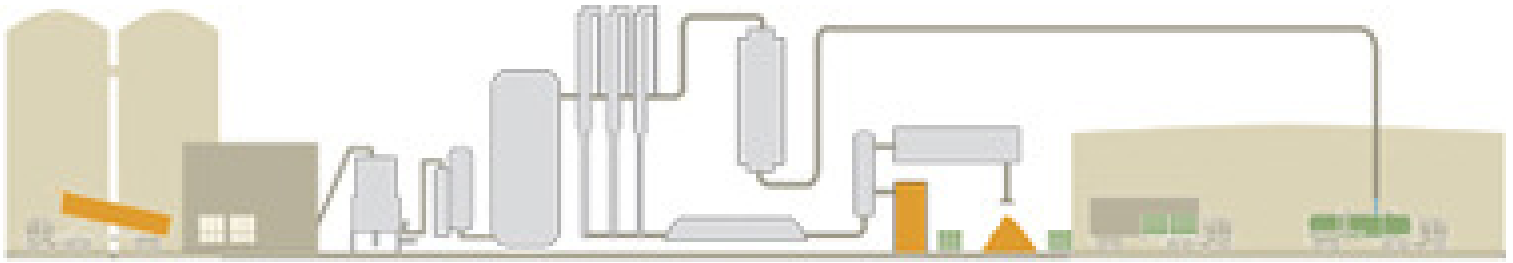
Current use of spectroscopy throughout ethanol production

FTIR for accurate fermentation monitoring

Summary

Ethanol Production
Different Needs at Different Control Points

FOSS

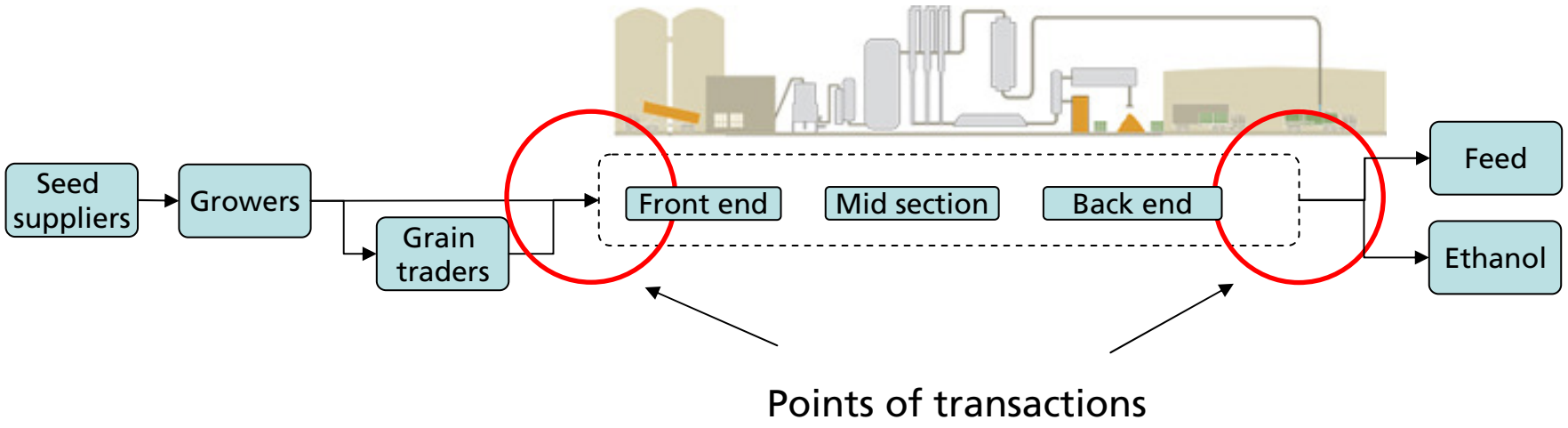


Front end | **Mid section** | **Back end**

Differences in
- process type
- reason to analyze
- frequency of analysis
- sample type
- locations
- personnel (access, skills)

} different analytical requirements !

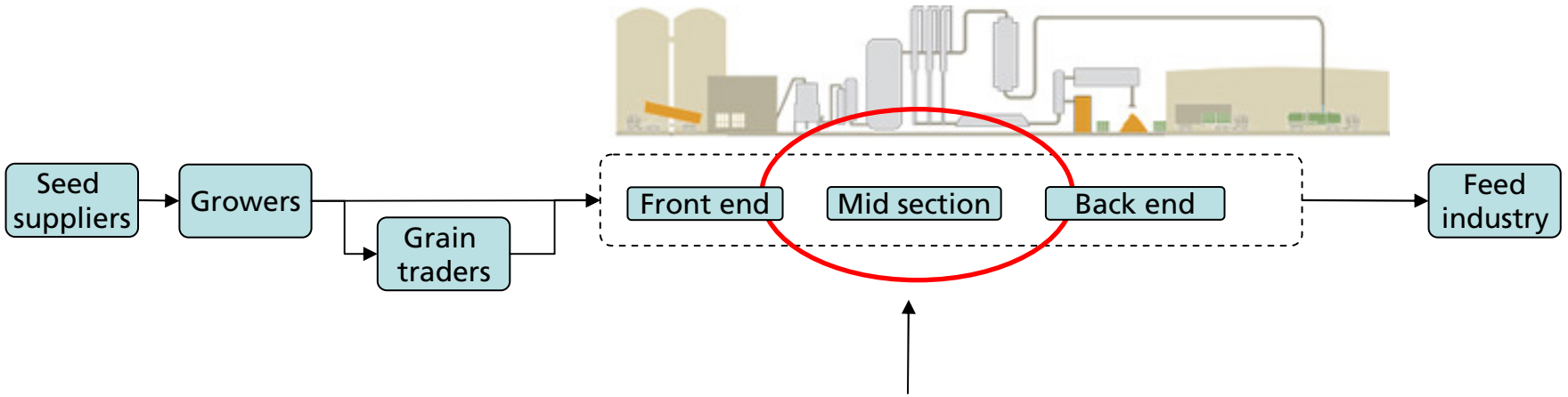
Grain Value Chain



Analyses at a plant must also match the need of suppliers and customers to ensure a good client-to-client relation based on fair and transparent systems.

Measurements at front and at back are dual purpose
– process optimization and payment

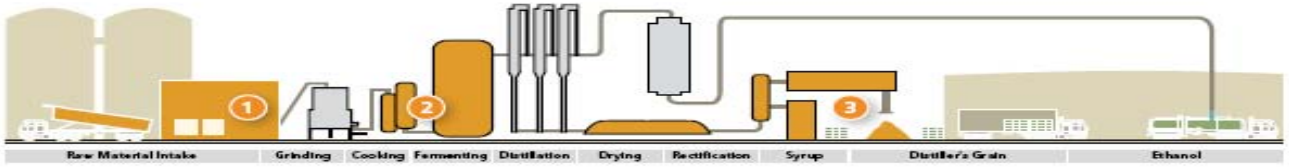
Grain Value Chain



Internal control for process optimization
(increase yield; reduce costs)

NIR for
Front End and Back End

FOSS



Front-end
Whole grain intake

Mid
Fermentation and mash/slurry

Back-end
DDGS and solids control

?

NIR

For intake control
- Robust and fast
- Approved for transaction analysis – by branches and governments (e.g. NTEP, GIPSA)

Parameters
- Ethanol Yield Potential
- Starch
- Protein
- Moisture
- Fat
- Test weight

NIR

For co-products
- Robust and fast
- Versatile to match all co-products
- Tie-in with feed companies QC-systems

Parameters
- Moisture
- Protein
- Fat
- Ash
- Fiber
- Amino Acids

Specific analytical requirements in Mid section / Fermentation

FOSS

Front end | Mid section | Back end

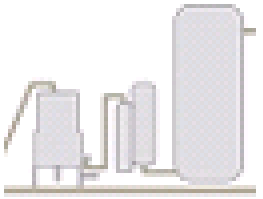
Internal process control

- Reproducibility / Accuracy
- Fast response time is crucial for process control
- Multi parameter
- Low level parameters (i.e. Lactic and Acetic Acid)

Samples are live (yeast / enzymes) and must be analyzed a.s.a.p.

- No long transportation of sample
- No waiting for busy instrument

Relying on the front-end grain intake analyzer for fermentation monitoring is not recommended.



A dedicated analyzer is required for this area !

Agenda

FOSS

Introduction to FOSS

Current use of spectroscopy throughout ethanol production

FTIR for accurate fermentation monitoring

Summary

FOSS' history and expertise in FTIR has opened up for new possibilities

- First mid IR systems in 1970's
- FOSS units officially approved globally
- 90% + of global milk analyzed on FOSS units
- Market leader in Dairy and wine
- FTIR is core technology

FOSS FTIR technology is now applied to Biofuels

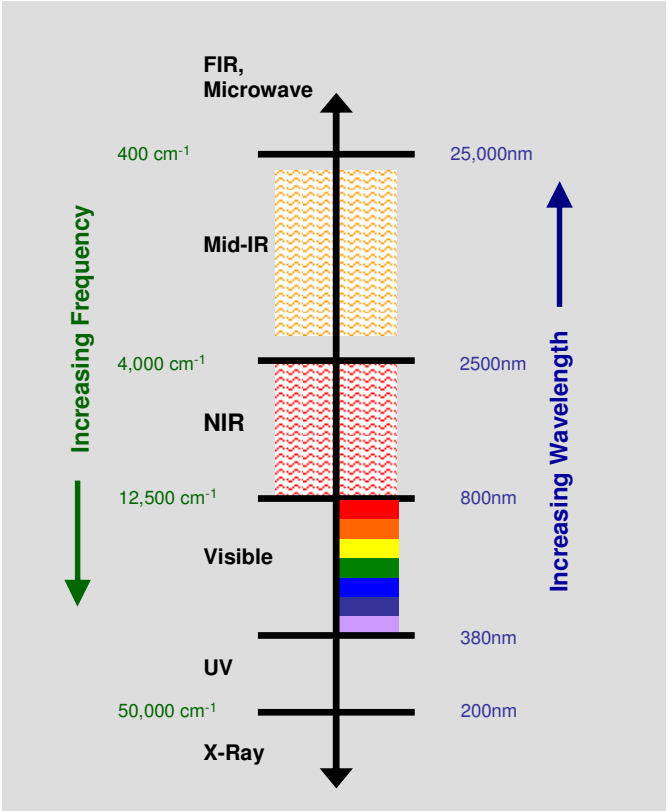
FTIR vs. NIR - theory

FOSS

FTIR = Fourier Transformed InfraRed

NIR = Near InfraRed

- *NIR = 400 – 2500 nm*
- *Mid-IR = 2500 nm – 25000 nm*
- *FT = Fourier Transform, is the mathematical technique used for transforming the detector output into a frequency spectra.*

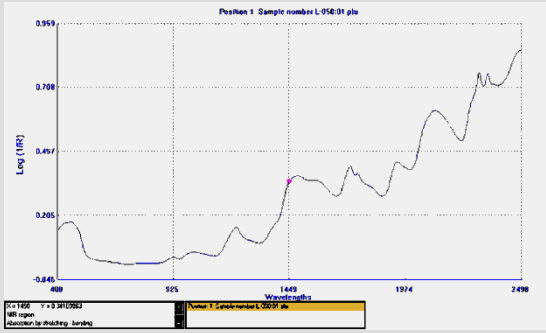


FTIR vs. NIR

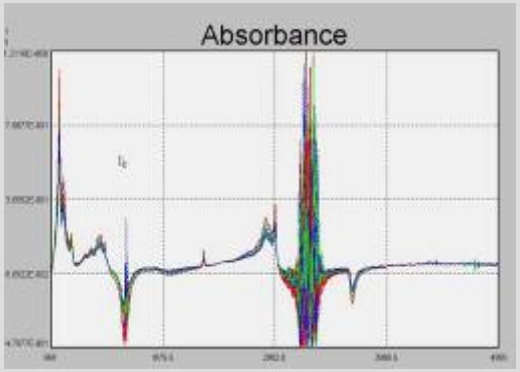
FOSS

- NIR spectroscopy is looking at the less absorbing overtones in the lower area of the spectra.
- The NIR spectra is broader, limiting the resolution of the analytical results. This can to some extent be overcome with powerful chemometric tools.
- In mid-IR spectroscopy, the absorbency bands are much sharper and separated thus more specific for individual components.
- Mid-IR is looking at the fundamental, strongly absorbing wavelengths thus giving lower detection limits.

NIR



FTIR



NIR vs. FTIR - features

FOSS

	NIR	FTIR
Speed	√	√
Easy to learn	√	√
Easy to use	√	√
Multiple parameters	√	√
No chemicals or pollutants	√	√
Safe	√	√

FTIR spectroscopy

FOSS

- Higher absorbency in FTIR range than in NIR range
  - FTIR uses smaller path lengths
  - Works well for liquids
    - *Not suitable for transmission in solids*
- Fundamental spectral response is found in the mid-IR range while NIR looks at overtones
  - FTIR thus more sensitive to low level constituents
- Experience from using FTIR in other areas shows that the acids we're looking for can be analyzed at low levels.

FTIR is in theory a better suited technology for fermentation monitoring than NIR

NIR vs. FTIR
- accuracy

FOSS

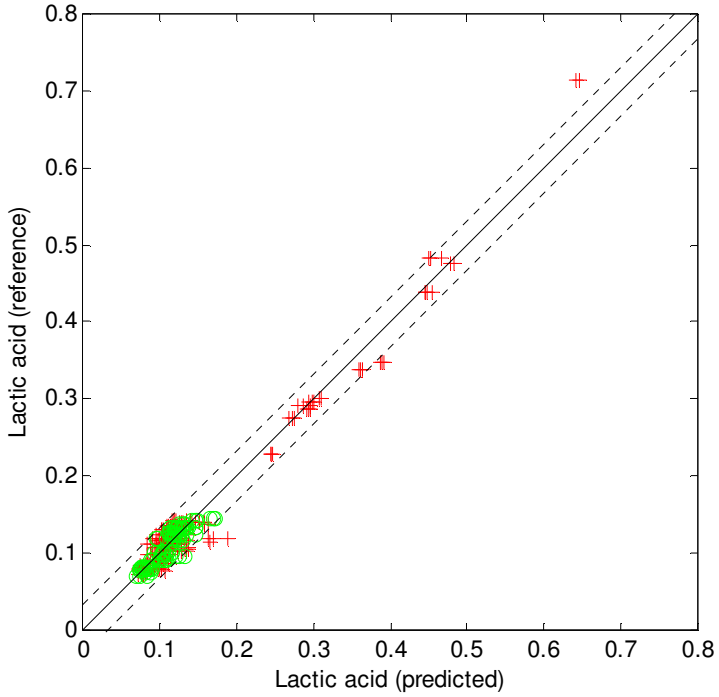
	Range	FOSS XDS NIR validation result (SEP)	BioFoss FTIR validation result (SEP)
Acetic acid *	0.00 – 0.25 %	>0.05	0.005%
Lactic acid *	0.07 – 0.72 %	>0.15	0.011%
Ethanol	5.82 – 12.80 %	0.186%	0.149%
DP4+	0.57 – 9.99 %	0.421%	0.144%
DP3	0.05 – 0.50 %	0.033%	0.032%
DP2 / Maltose	0.28 – 2.59 %	0.179%	0.071%
Glucose	0.01 – 6.24 %	0.257%	0.119%
Glycerol	0.82 – 1.36 %	0.021%	0.014%
pH	3.18 – 4.08	0.076	0.044

SEP = Standard Error of Prediction

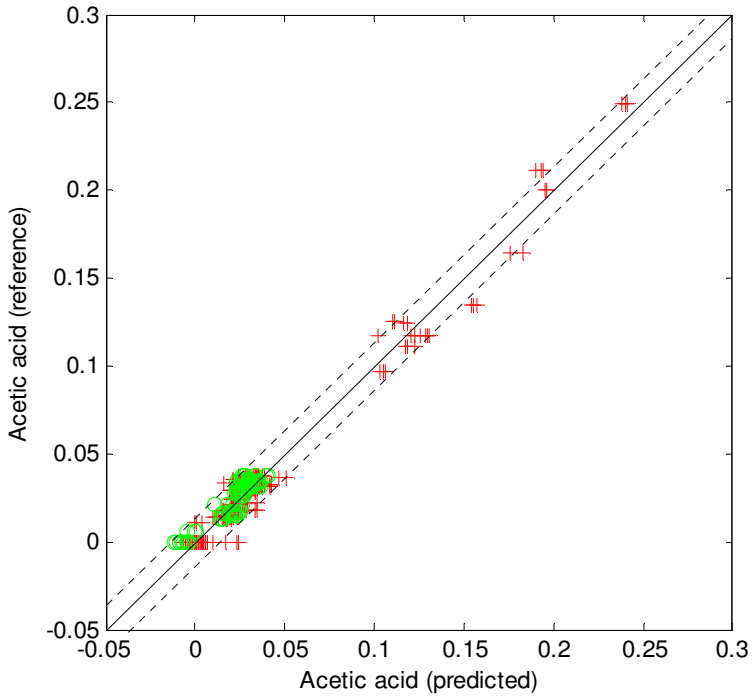
* The acids has a very strong correlation with ethanol and may show a seemingly good (but false) SEP value if these internal correlations aren't dealt with during calibration development.

Lactic & Acetic Acid
- performance using FTIR

FOSS



Lactic		(% w/v)
SEP	0,011	
S_r	0,005	



Acetic		(% w/v)
SEP	0,005	
S_r	0,002	

Agenda

FOSS

- **Introduction to FOSS**
- **Current use of spectroscopy throughout ethanol production**
- **FTIR for accurate fermentation monitoring**
- **Summary**

Conclusion regarding Fermentation monitoring using FTIR

FOSS

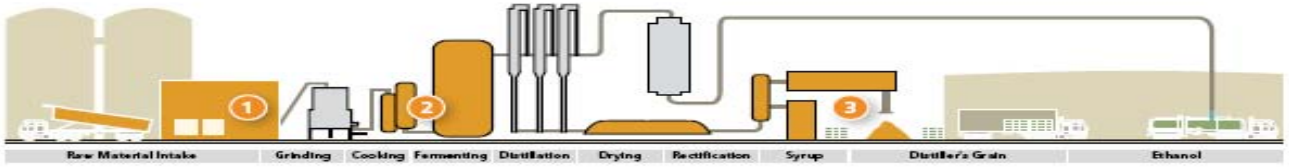
- FTIR performance is generally better than NIR for fermentation parameters

- Only FTIR has the capabilities to determine the infection parameters well enough
  - Acetic acid
  - Lactic acid

- A coarse filtration (paper filter) is required for FTIR
  - Does not allow for moisture analysis

BioFoss ™

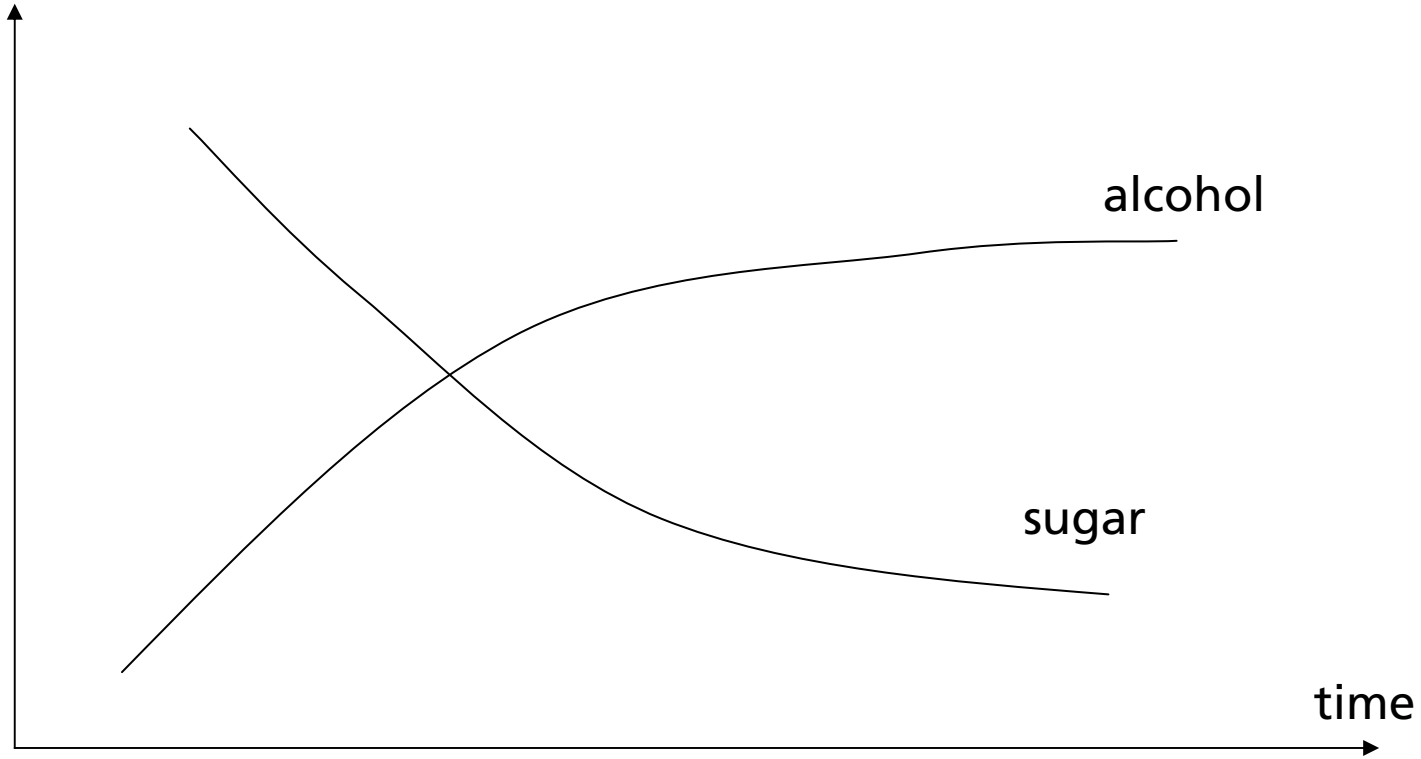
Summary - Ethanol solutions



Front-end Whole grain intake	**Mid** Fermentation and mash/slurry	**Back-end** DDGS and solids control
		
NIR	**FTIR**	**NIR**
For intake control • Robust and fast • Approved for the transaction	**For accurate fermentation** • Robust and fast • Sensitive enough for low level constituents • Very good performance on all relevant parameters	**For co-products** • Robust and fast • Versatile to match all co-products • Tie-in with feed companies QC-systems
Parameters • Ethanol Yield Potential • Starch • Protein • Moisture • Fat • Test weight	**Parameters** • Lactic Acid • Acetic Acid • Ethanol • Glucose • Maltose • DP3 • DP4+ • Glycerol • pH	**Parameters** • Moisture • Protein • Fat • Ash • Fiber • Amino Acids

FOSS

Natural correlations occur between components during a normal fermentation



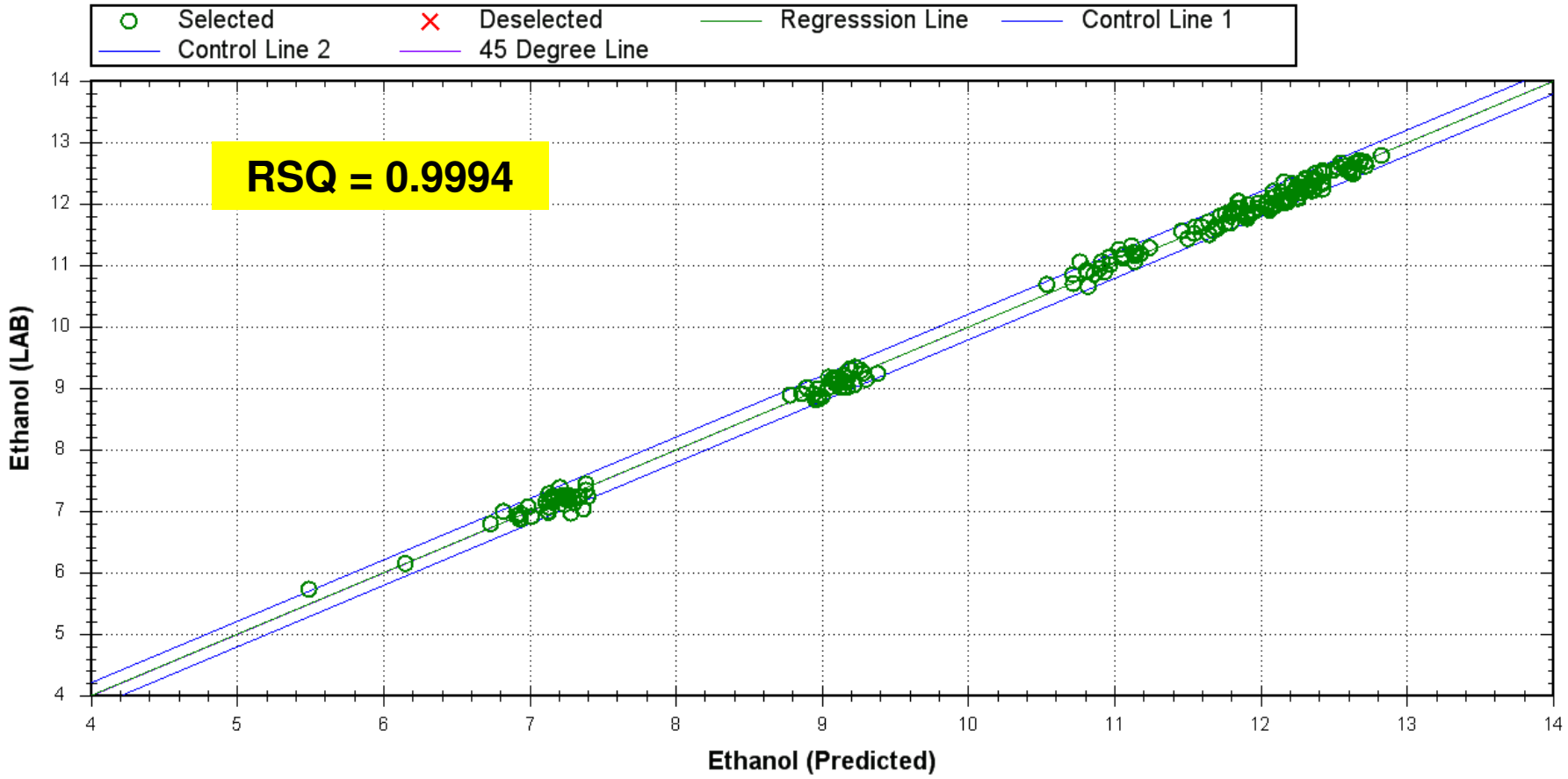
Normal case
– fault free fermentation

FOSS

Correlations between components based on reference (HPLC) analysis
> 100 samples

	pH	DP4+	DP3	Maltose	Glucose	Lactic	Glycerol	Acetic
pH	1.00							
DP4+	0.35	1.00						
DP3	0.49	0.83	1.00					
Maltose	0.41	0.90	0.94	1.00				
Glucose	0.01	0.28	0.03	0.06	1.00			
Lactic	0.47	0.78	0.91	0.88	0.04	1.00		
Glycerol	0.25	0.92	0.64	0.75	0.42	0.56	1.00	
Acetic	0.36	0.98	0.84	0.92	0.22	0.80	0.89	1.00
Ethanol	0.26	0.93	0.63	0.70	0.52	0.57	0.97	0.89

Ethanol is easy in NIR



Infected fermentation – spiked with Acetic and Lactic Acid

FOSS

Correlations between components based on reference (HPLC) analysis > 100 samples

	pH	DP4+	DP3	Maltose	Glucose	Lactic	Glycerol	Acetic
pH	1.00							
DP4+	0.30	1.00						
DP3	0.19	0.80	1.00					
Maltose	0.21	0.83	0.89	1.00				
Glucose	0.30	0.32	0.08	0.06	1.00			
Lactic	0.26	0.02	0.02	0.02	0.01	1.00		
Glycerol	0.18	0.85	0.57	0.61	0.36	0.01	1.00	
Acetic	0.06	0.09	0.07	0.08	0.03	0.01	0.08	1.00
Ethanol	0.37	0.87	0.55	0.56	0.65	0.02	0.84	0.08

Break the correlations

FOSS

Correlations between components based on reference (HPLC) analysis
> 100 samples

[Normal]

	pH	DP4+	DP3	Maltose	Glucose	Lactic	Glycerol	Acetic
Ethanol	0.26	0.93	0.63	0.70	0.52	**0.57**	0.97	**0.89**

[Infected]

	pH	DP4+	DP3	Maltose	Glucose	Lactic	Glycerol	Acetic
Ethanol	0.37	0.87	0.55	0.56	0.65	**0.02**	0.84	**0.08**