In-Situ Monitoring of Fermentation Reactions

Peter Melling and Mary Thomson
Remspec Corporation

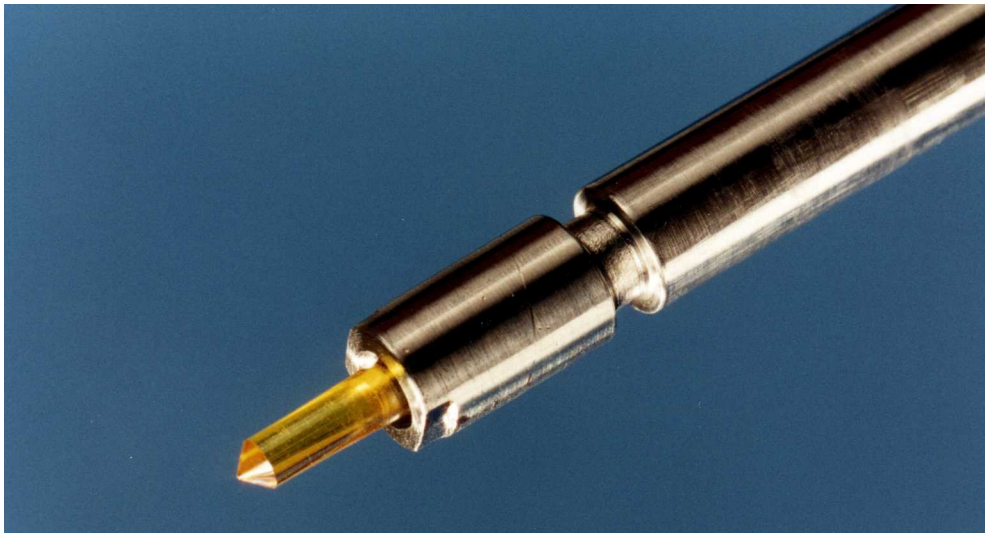
Fermentation Monitoring

- Mid-IR monitoring with ReactionView can measure:
  - Reaction progress
    - Disappearance of substrate (sugars etc.)
    - Appearance of product(s) (alcohols etc.)
  - “Health” of the reaction
    - Carbon dioxide formation
    - Nutrient consumption
    - Waste product formation

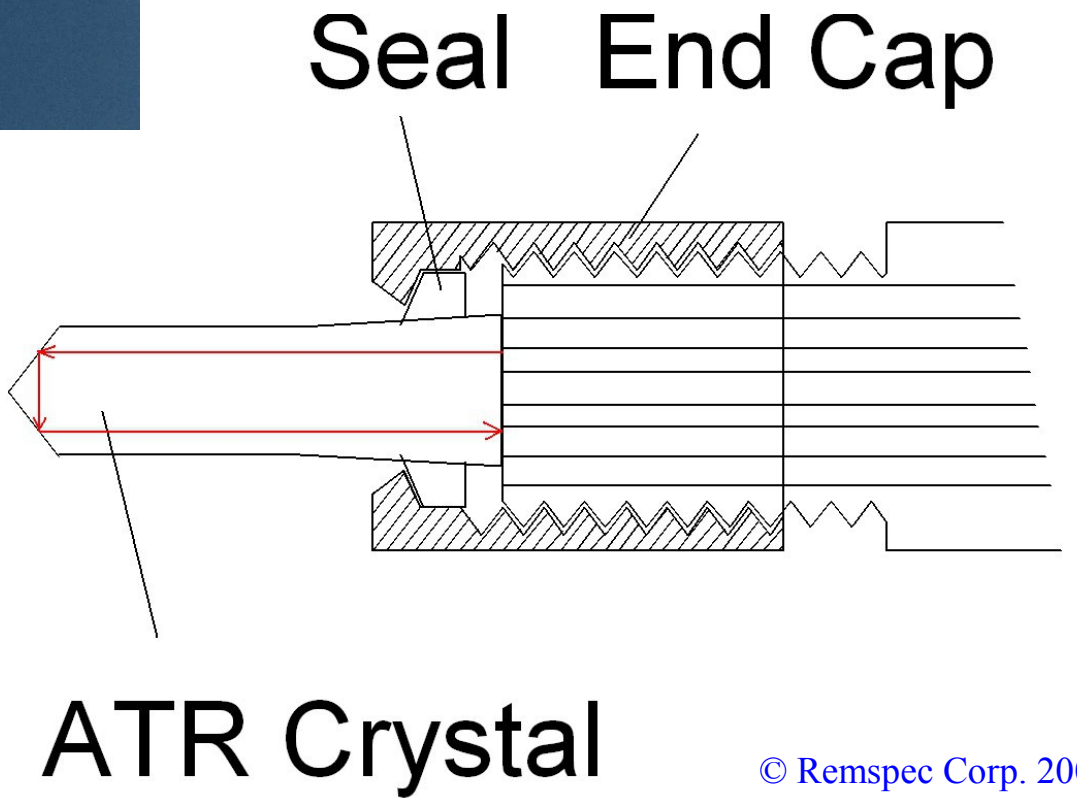
Fermentation Monitoring: Example

- Cloudy, aqueous medium with varying optical density (from yeast or other cell cultures)
  - ATR in the mid-IR samples only the liquid phase and is not affected by suspended solids or varying optical density

The ReactionView ATR Head

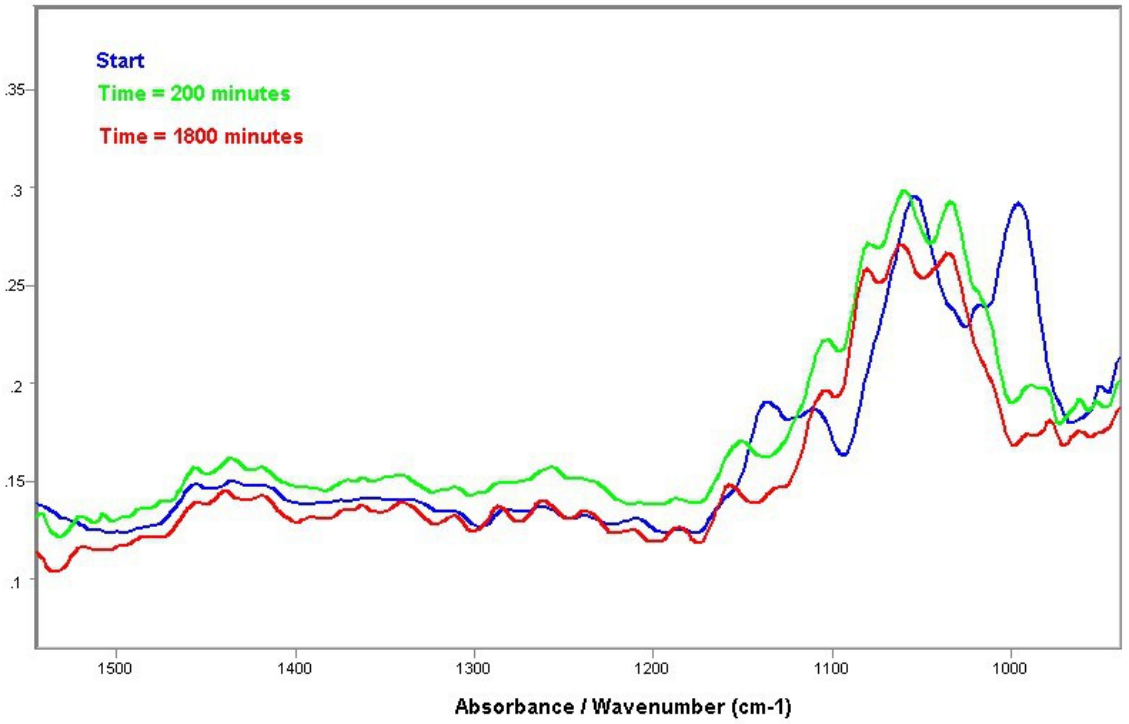


The ReactionView probe head is slim (6 mm diameter) and non-reactive. ATR crystals are made of ZnSe (shown) or ZnS.



The ATR crystal is a “2-bounce” design with excellent optical performance.

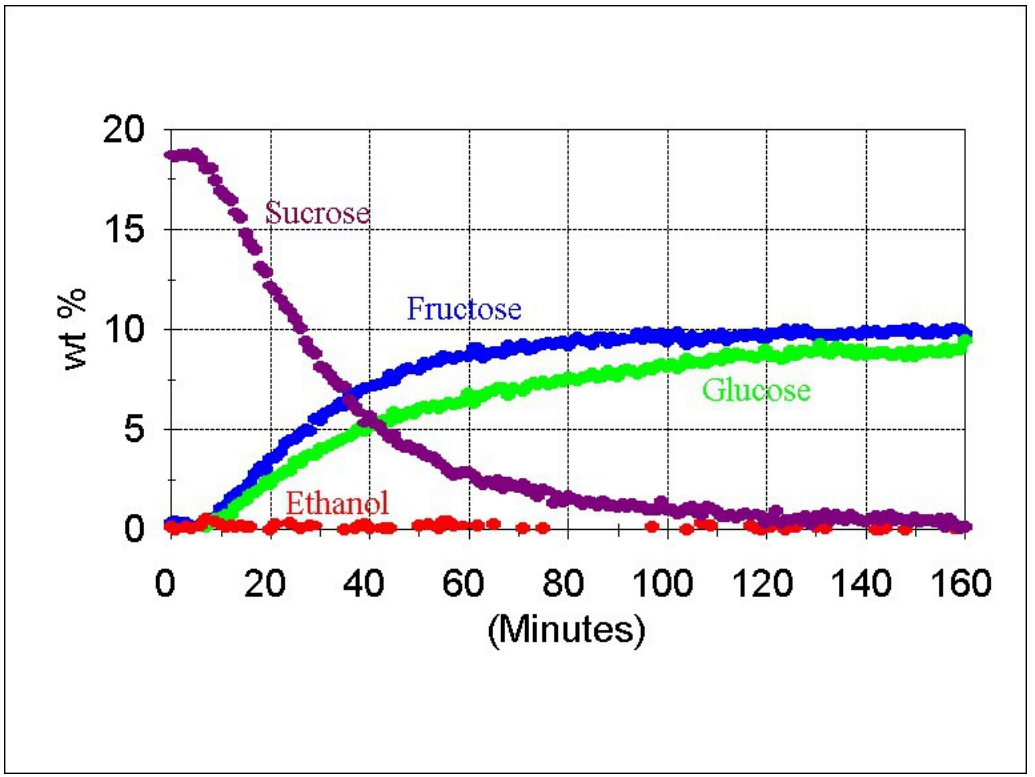
Fermentation of Sucrose: *S. cerevisiae*



- Starting spectrum (blue) characteristic of sucrose
- Changes slowly to a mixture of fructose and glucose, finally ethanol
- Statistical ("chemometric") methods needed to interpret sugar spectra

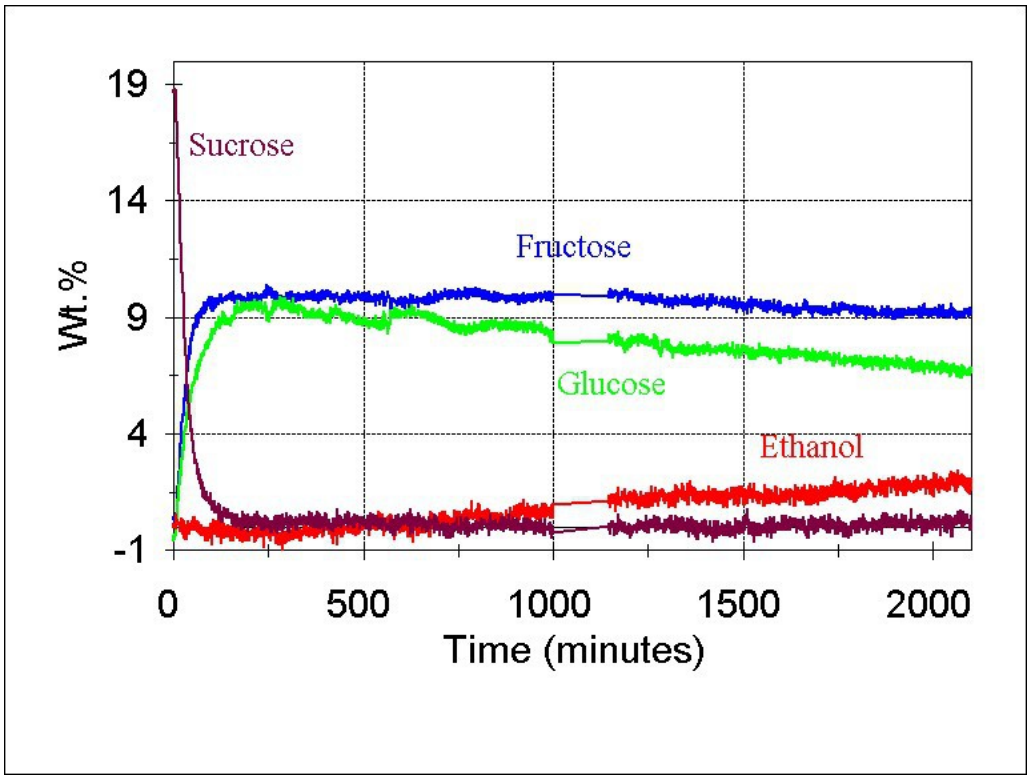
[published in *Spectroscopy,* June 2001]

Fermentation of Sucrose:
First 2 hours, inversion



- A simple PLS analysis gives concentration/time curves for the sugars during inversion (conversion of sucrose into fructose and glucose)

Fermentation of Sucrose:
After 2 hours, glucose → ethanol

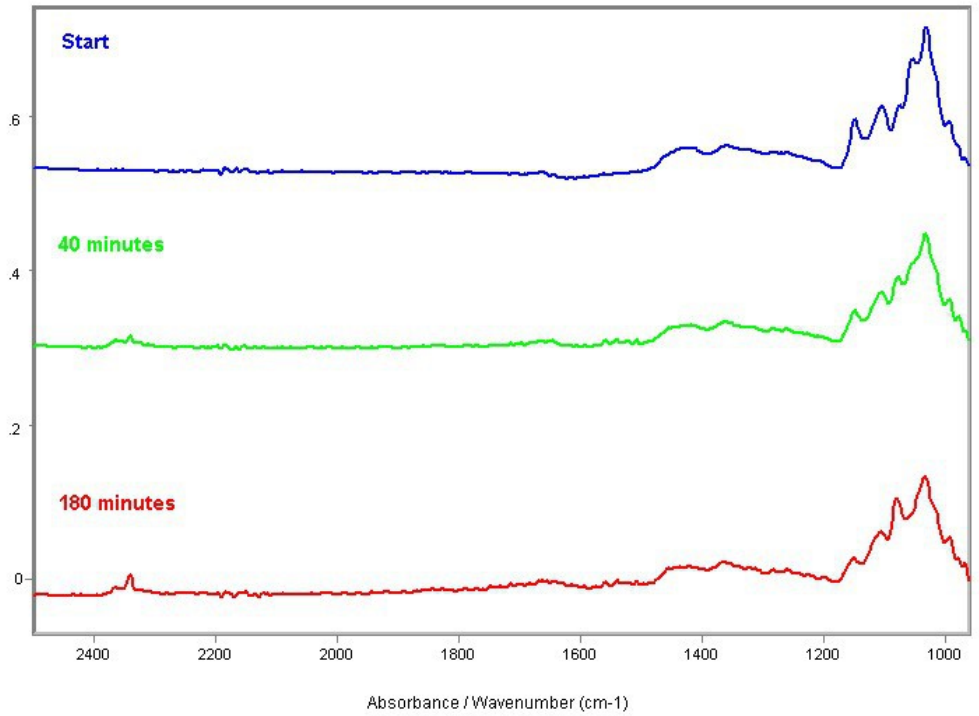


- After several hours, the ethanol spectrum begins to develop and can also be tracked (red line).

Fermentation Monitoring: Example

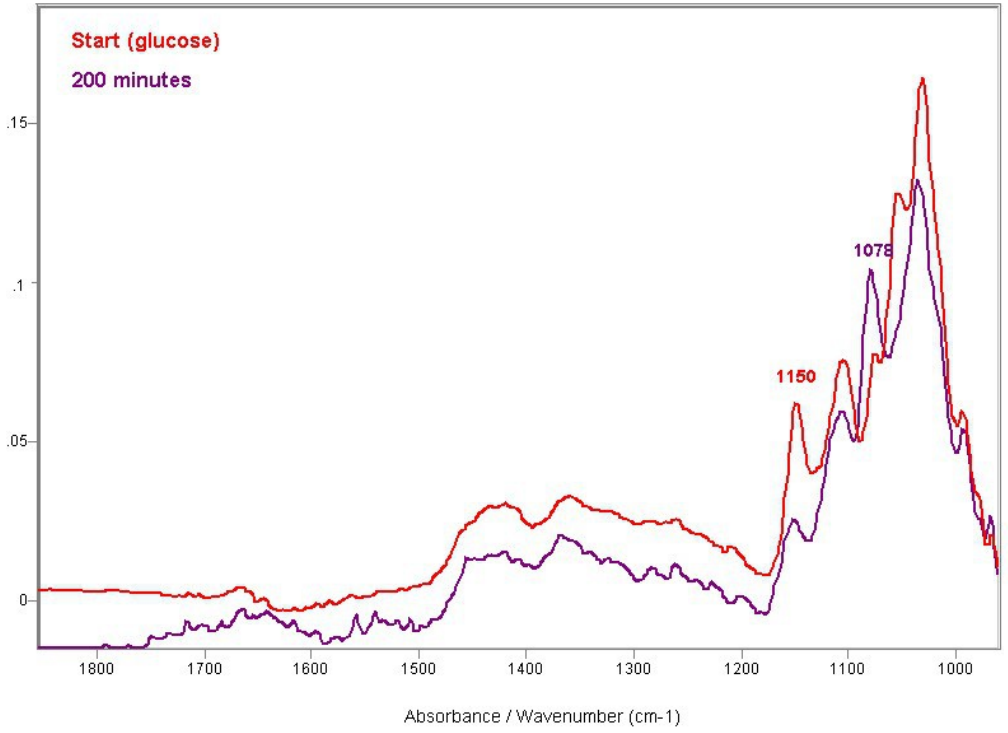
- Water
- Glucose
- Two strains of *Saccharomyces cerevisiae*
  - Household bread yeast
    - Optimized for rapid working, CO_2 production
  - Wine yeast (champagne)
    - Optimized for slower working, ethanol production

Fermentation of Glucose:
bread yeast



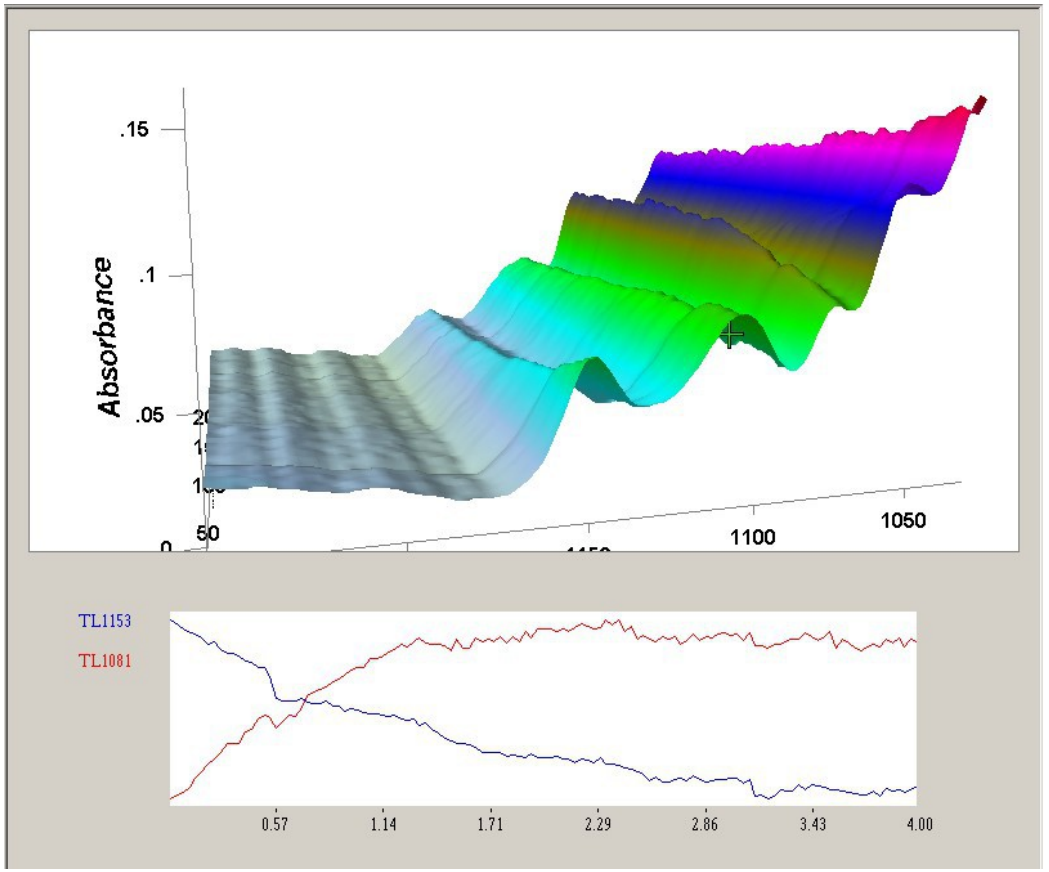
- In the sugar region (1000-1300 cm^{-1}), the glucose spectrum begins to diminish, and ethanol emerges.
- A distinct peak emerges in the CO_2 region (2300-2400 cm^{-1}).

Fermentation of Glucose:
bread yeast



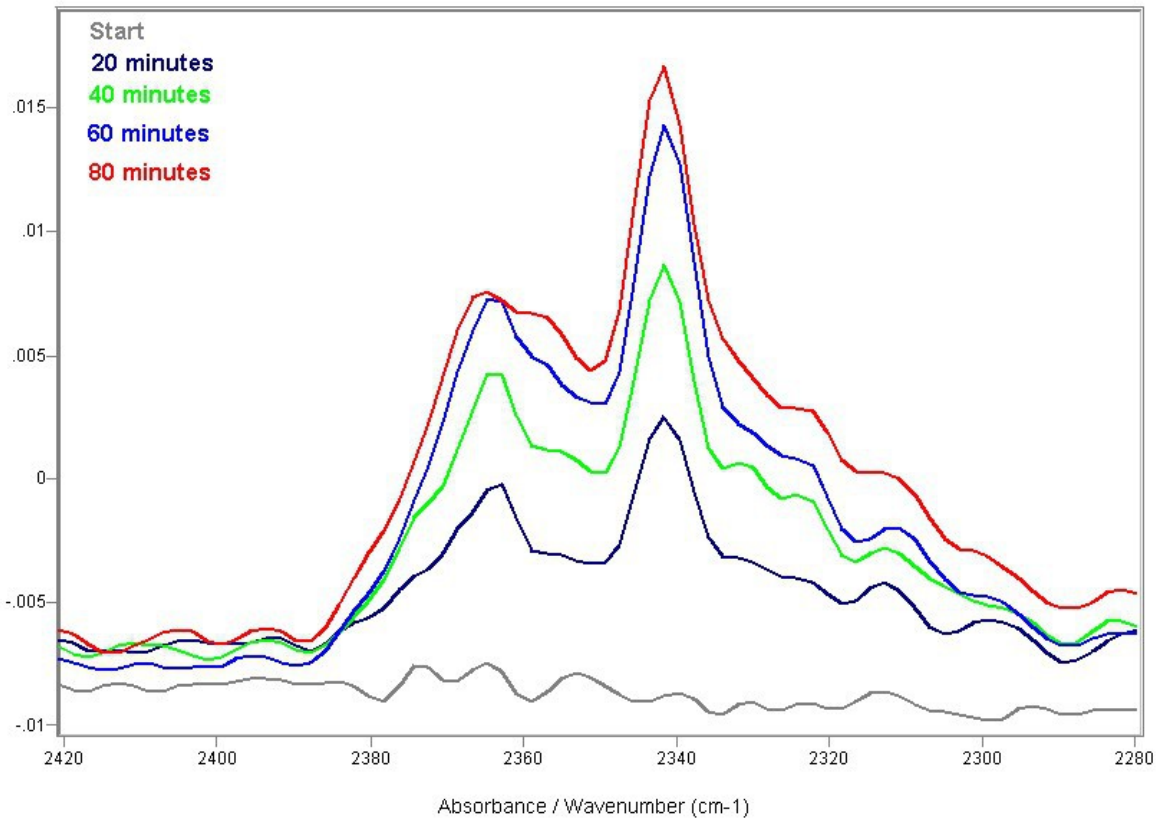
- By comparing the first and last spectra, areas of change can be identified (see marked peaks at 1078 and 1150 cm^{-1}).

Realtime Display of Mid-IR Spectra:
Glucose/ethanol region



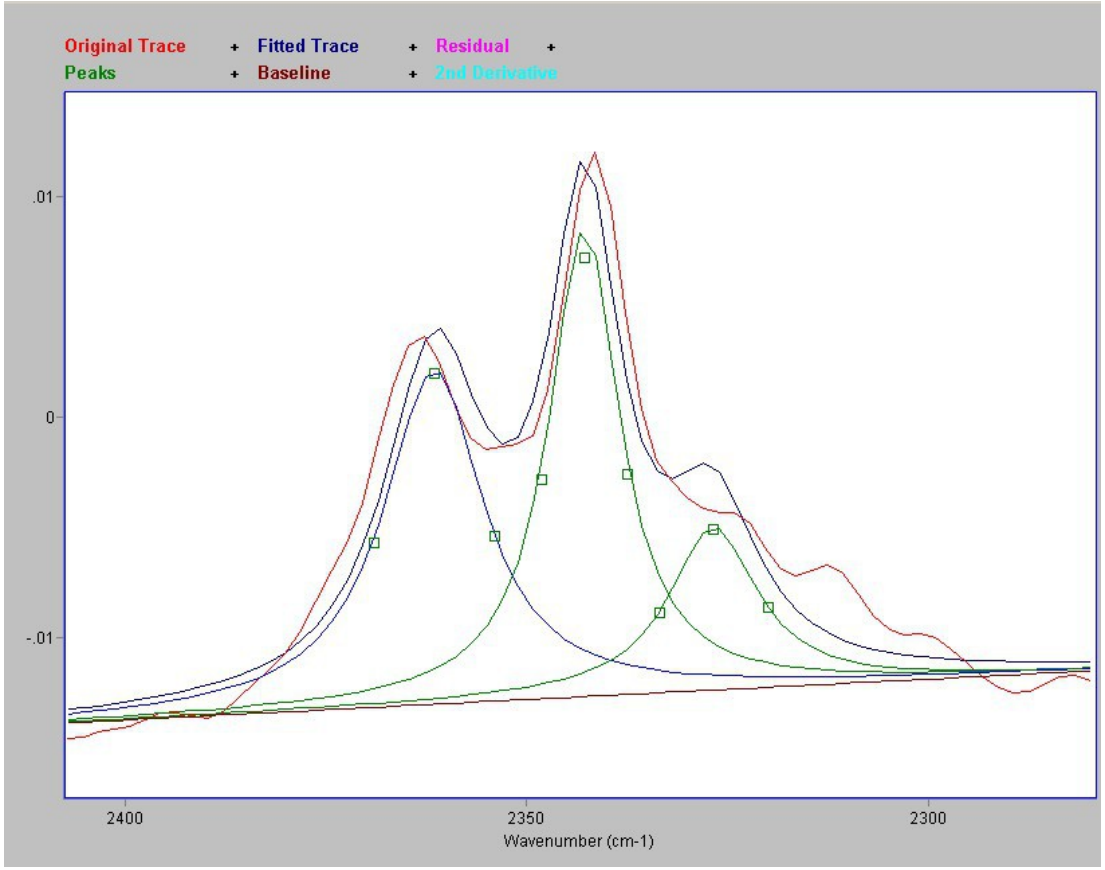
- The changes with time are clearly visible in the realtime 3D display provided in VizIR™.
- Simple trendlines at the selected frequencies give a quick visual guide to the progress of the reaction.

Mid-IR Spectra:
Carbon dioxide region



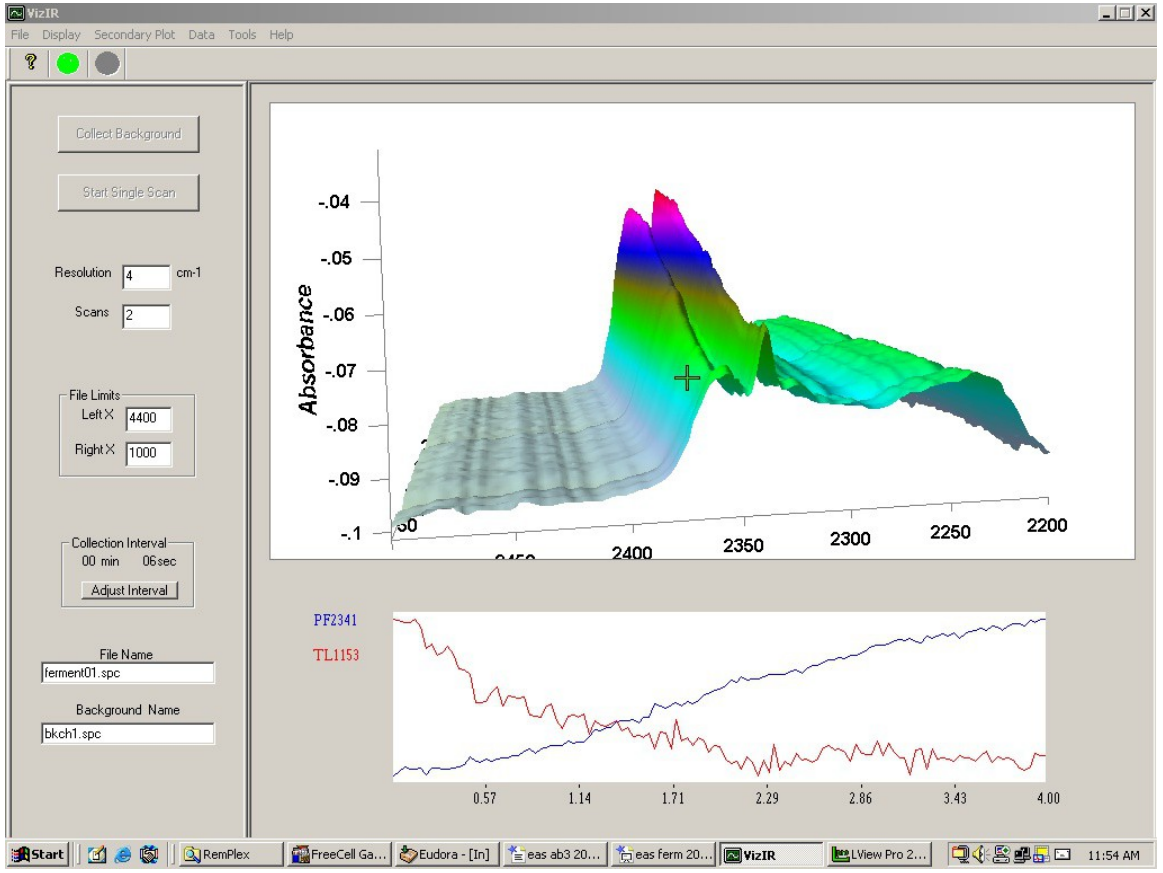
- A single peak emerging at 2342 cm^{-1} is characteristic of dissolved CO_2, and a robust calibration has been developed.
- The neighboring peaks are associated with other forms of CO_2, e.g. contained in small bubbles; these features are hard to calibrate.

Mid-IR Spectra:
Carbon dioxide region



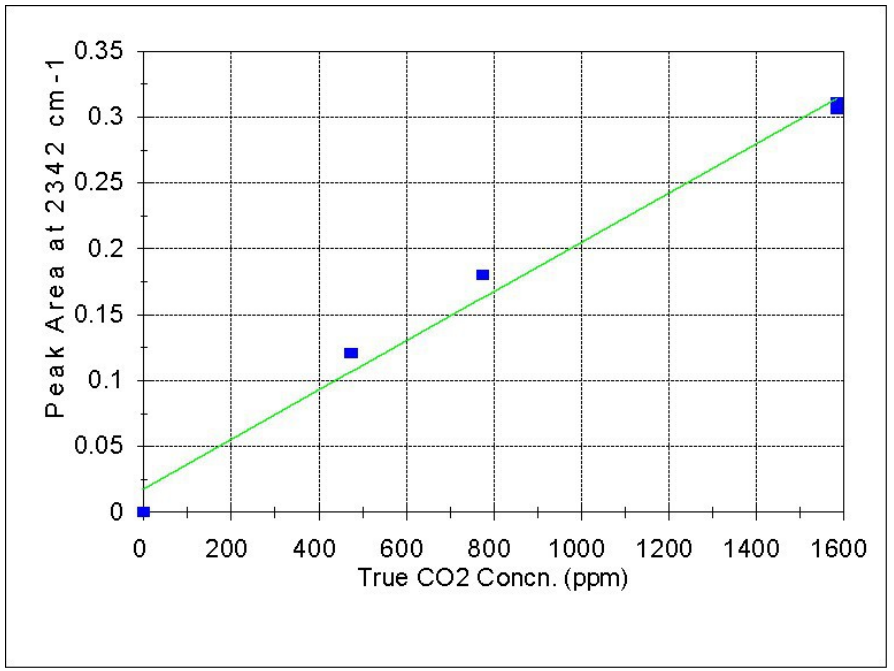
- The calibration is based on a peak-fitting model of the CO_2 spectral region.
- VizIR™ can run the model in real time as spectra are collected, to generate a trendline.
- The peak at 2342 cm^{-1} can be calibrated against known dissolved CO_2 concentrations.

Realtime Display of Mid-IR Spectra:
Carbon dioxide region



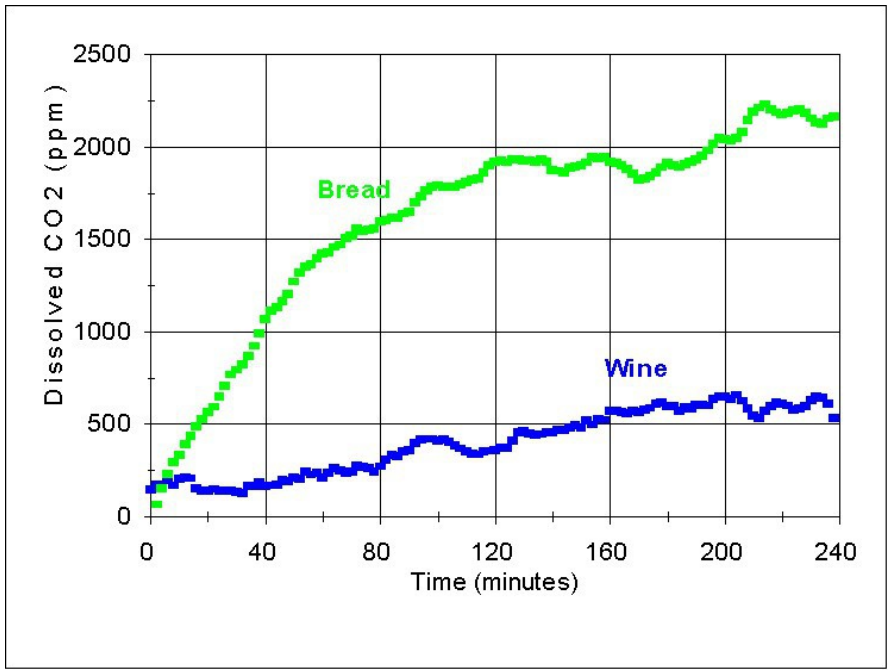
- The changes in the CO_2 region are clearly visible in the 3D display.
- The CO_2 trendline is based on the peak at 2342 cm^{-1} from the peak-fit model, and is combined with a crude intensity/time trace for a glucose peak.

Mid-IR Spectra:
Calibration of Trendlines



- Calibration standards prepared by dissolving sodium bicarbonate in water at pH 4
  - Gives quantitative dissolved CO_2 up to ~2000 ppm.
- Using the peak area at 2342 cm^{-1}, a straight-line calibration plot is developed
  - Can be extrapolated

Mid-IR Spectra:
Calibration of Trendlines

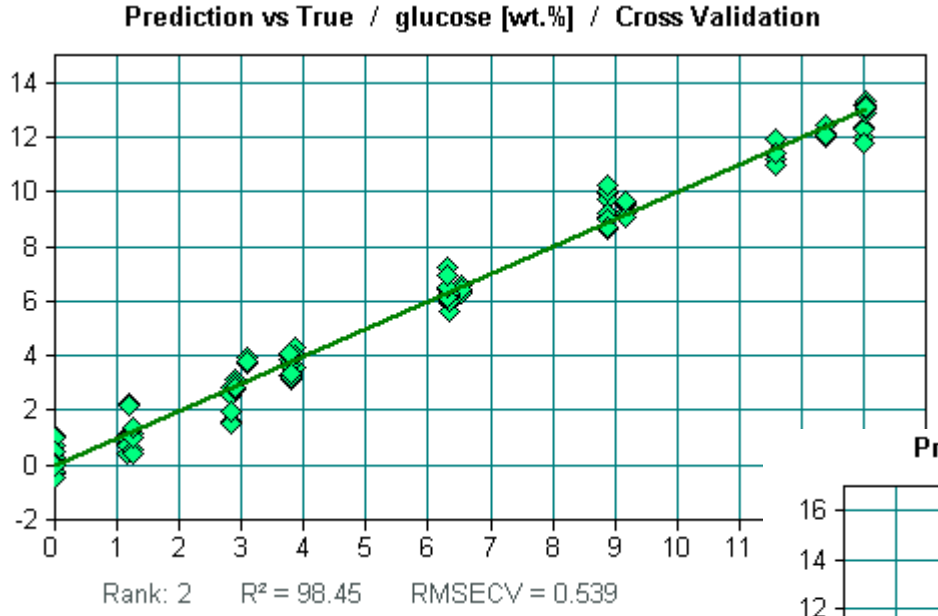


- The CO_2 peak area is converted to ppm and graphed against time.
- As expected, the bread yeast (green line) evolves CO_2 faster than the wine yeast (blue line) over the first four hours of the reaction.
  - Both reactions were run at 23°C.

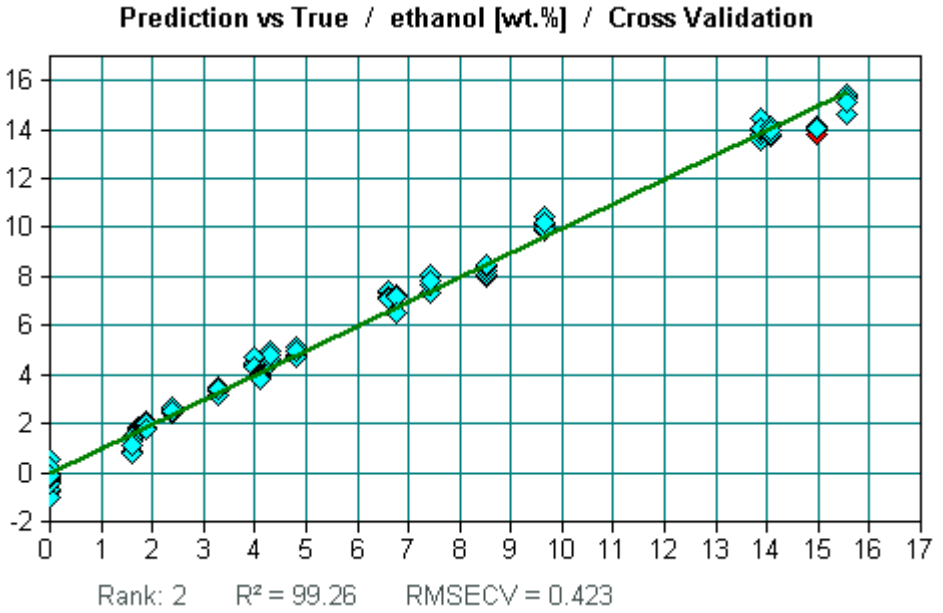
Mid-IR Spectra:
Calibration of Trendlines

- A chemometric software package was used to carry out a simple calibration for glucose and ethanol, with a series of 24 aqueous mixtures of glucose and ethanol.
- The CO_2 was calibrated using the area of the peak at 2340 cm^{-1}, as calculated from the peak-fit model.

Mid-IR Spectra:
Calibration of Trendlines

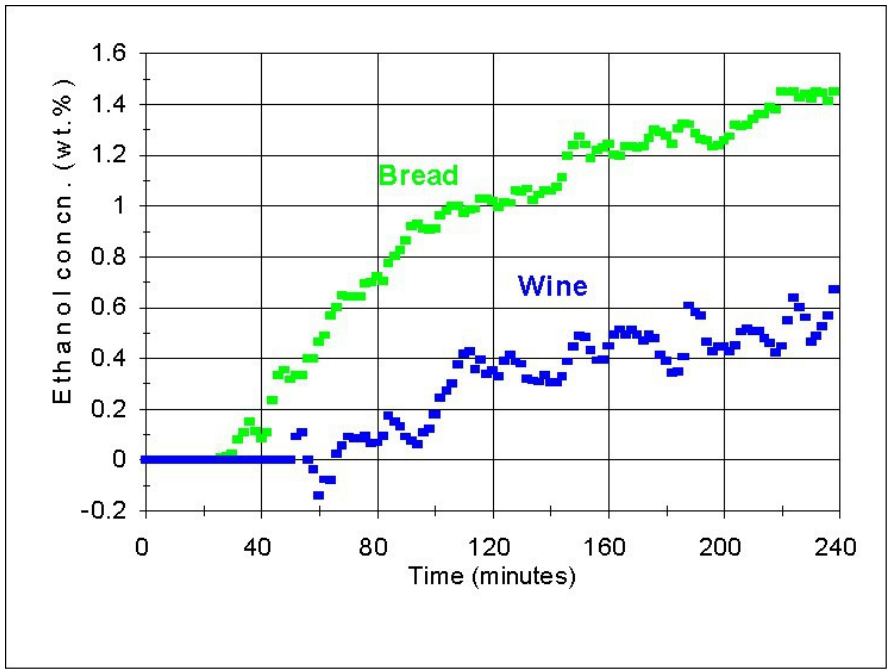


Glucose



Ethanol

Mid-IR Spectra:
Calibration of Trendlines



- Quantitative results (in wt. %) for ethanol are plotted against time.
- The bread yeast (green line) develops ethanol faster than the wine yeast over the first four hours of the reaction.
- The wine yeast works more slowly, and the ethanol concentration reaches only ~0.5 wt.% in four hours.

Conclusions

- ReactionView with an ATR head provides reliable, quantitative spectral information in realtime from fermentation reactors.
- The spectra derive from the dissolved species, without interference from the biomass.
- Substrates and products can be tracked using simple intensity/time curves in many cases, or using chemometric methods. All of these methods can be used in real time.
- Dissolved CO_2 has been calibrated based on a peak-fit model to give reliable results that are transferable among systems.