Fermentation Process Monitoring: Dissolved Carbon Dioxide

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Figure 1: Fermentation reactor with ReactionView probe

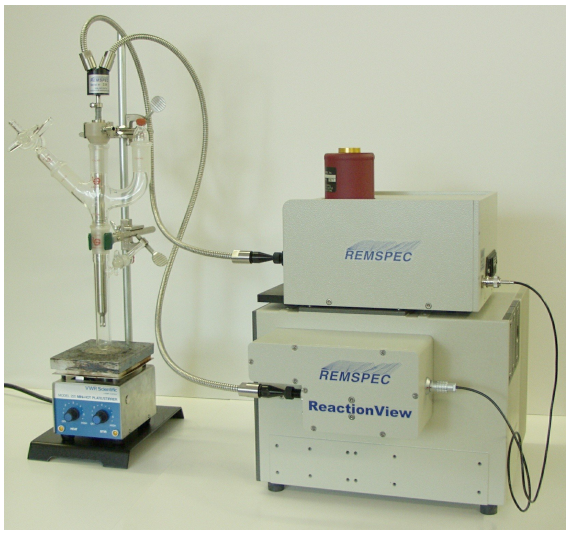


Figure 2 Single-channel ReactionView System

Figure 3: Close-up of the zinc sulfide ATR head

The monitoring and control of carbon dioxide levels inside reactors is important in numerous areas of biotechnology and pharmaceutical chemistry. In fermentation reactions, carbon dioxide levels can give an important indication of the progress of a reaction, and of the overall health of the fermenting medium. Certain antibiotics are solubilized by treatment with carbonates, and the end point can be determined quickly and easily by monitoring the level of dissolved carbon dioxide. Many conventional approaches to this problem rely on monitoring off-gases or headspace gases for carbon dioxide content, giving an indirect measure of dissolved gas with a built-in time lag.

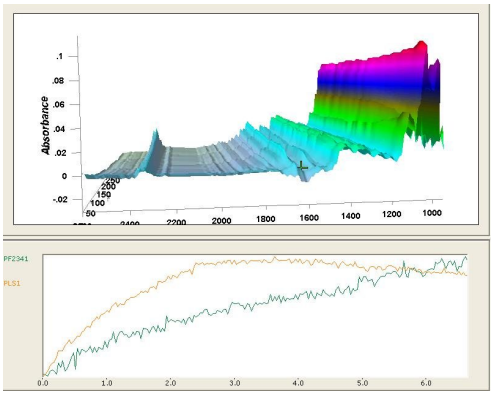
In-situ mid-IR spectroscopy offers real-time, direct measurement of dissolved carbon dioxide in aqueous media, including opaque fermentation broths and reactors with suspended solids content. The ReactionView® offers true mid-IR spectroscopy based on a compact FTIR source and a laptop computer. Flexible IR-transmitting fiber bundles and a variety of sampling heads mean that this system is suitable for a wide range of reactor types and conditions.

Monitoring a Fermentation Reaction: Sucrose and *Saccharomyces cerevisiae*

Experimental

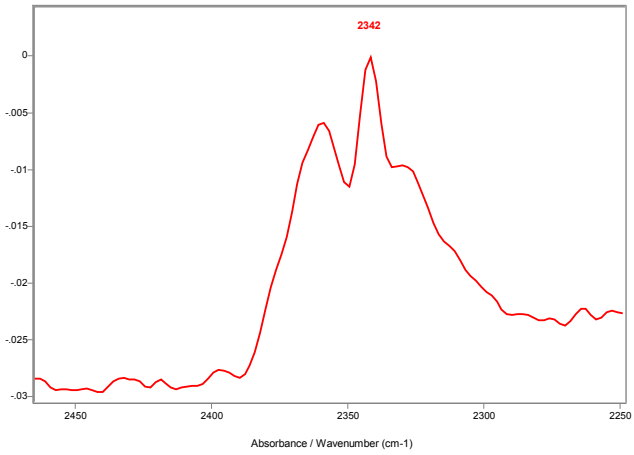
A stirred glass reactor (1L capacity, see Fig. 1) was charged with water (approx. 600 mL). A ReactionView mid-IR probe fitted with a zinc sulfide ATR crystal (overall probe diameter 6 mm) was fitted into one of the unused 24/40 ground glass fittings in the top or the reactor using a standard thermometer fitting so that the ATR crystal was immersed in the water. A background spectrum was obtained, then sucrose (60 g) was added. After the sucrose had dissolved, data collection was started: the FTIR spectrum of the reaction mixture was recorded every 2 minutes (collection time 30 seconds per spectrum, resolution 4 cm^{-1}, spectral range 900-5000 cm^{-1}). After the first spectrum was complete, 10 mL of a yeast solution (prepared from domestic dried baker's yeast, glucose, and water) was added and spectra were collected for a total of 7 hours.

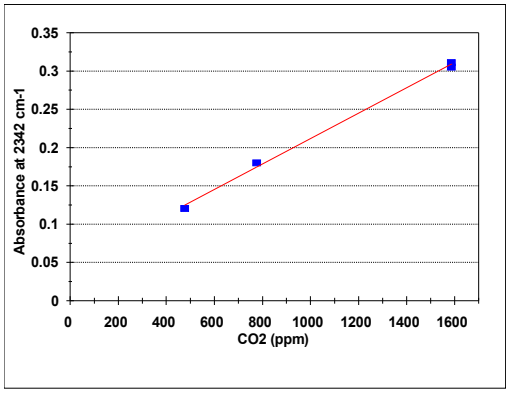
Results



VizIR™ is the reaction monitoring software used by ReactionView®. This screen shot, taken towards the end of the fermentation, shows a 3D plot of the spectra collected during experiment, and two trendlines based on changes in the spectra. The green trendline shows the growth of a prominent peak at 2342 cm^{-1} assigned to dissolved carbon dioxide, and calibrated using a peak-fit model. The orange trendline shows the formation and subsequent disappearance of glucose, based on a PLS1 calibration model. Both calibration models were run in real time.

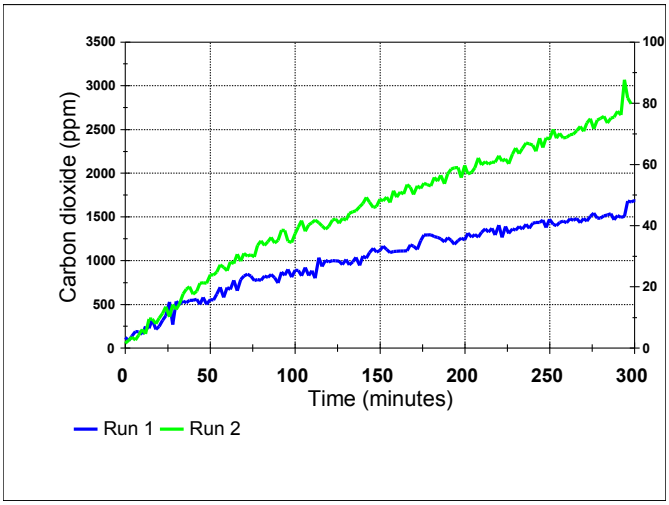
This expanded section from one of the spectra obtained near the middle of the experiment shows the carbon dioxide peaks in the spectrum. The single peak at 2342 cm^{-1} is a stretching mode of dissolved carbon dioxide. The two peaks on either side arise from gaseous carbon dioxide which is effervescing out of the solution. A simple peak fitting routine can be implemented in real time by VizIR to calculate the area of the peak at 2342 cm^{-1} and the results were used for the trendline.

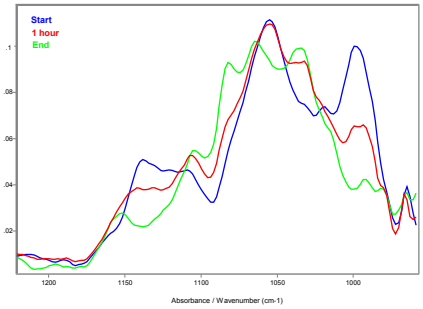




The peak area at 2342cm^{-1}, as determined using peak fitting, can be calibrated against standards (prepared in this case using buffered sodium bicarbonate solutions). This has proved to be a very robust calibration and has been successfully used to quantitate dissolved carbon dioxide in fermenters and in studies of the solubilization of antibiotics using aqueous carbonate salts.

When the same fermentation reaction was repeated at a different temperature (Run 1 was at 67°F and Run 2 was at 72°F), a marked change was observed in the build-up of dissolved carbon dioxide. A number of competing factors may influence this: rates of sucrose inversion and glucose consumption by the yeast tend to increase with temperature, while the solubility of carbon dioxide in water decreases with temperature.





The sugar region of the mid-IR spectra of the reaction mixture is shown on the left, at three different times during the reaction. The initial (blue) spectrum is that of sucrose, and the final (green) spectrum is a combination of the spectra of fructose and glucose arising from the sucrose inversion. The one-hour spectrum (red) is a combination of all three sugar spectra. The disappearance of sucrose can be crudely tracked using the peak at 998 cm^{-1}, but the complete analysis of the evolution of the sugar spectra and the emergence of the ethanol spectrum after long reaction times requires chemometrics[1] and the ability to use PLS models in real time is extremely valuable in the case of well-understood reactions where minor changes in conditions can have a considerable impact on the kinetics.

Discussion

In-situ mid-IR spectroscopy with ReactionView® opens up a chemical "eye" inside a fermenter or other reactor to measure dissolved carbon dioxide directly and in real time. It is no longer necessary to rely on indirect headspace or off-gas measurements. In addition, the whole of the mid-IR spectrum is available. This means that other chemical changes can be monitored and correlated with the carbon dioxide measurement. In many cases, it can be shown that the carbon dioxide measurements can be correlated with other parameters such as substrate consumption (in this case, the sucrose) and yeast growth[2]. Thus, in-situ FTIR provides an ideal and robust tool for fermentation monitoring.

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1 Al Mosheky, Melling and Thomson,*Spectroscopy,*June 2001
2 Pandiell et al., *Tech. Q. Master Brew. Assoc. Am.,* 1995, 32(3), 126-131