Fermentation Monitoring by In-Situ Mid-IR Spectroscopy

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Fermentation reactions are among the oldest chemical processes used on an industrial scale, and are central to the modern biotechnology. In this work, fiber-optic mid-IR spectroscopy is used to detect and track the concentration of the reactants and products in a simple fermentation reaction using a glucose substrate and commercial baker's yeast *(Saccharomyces cerevisiae)*. Glucose consumption, ethanol production, and carbon dioxide (CO_2) formation are all tracked in real time.

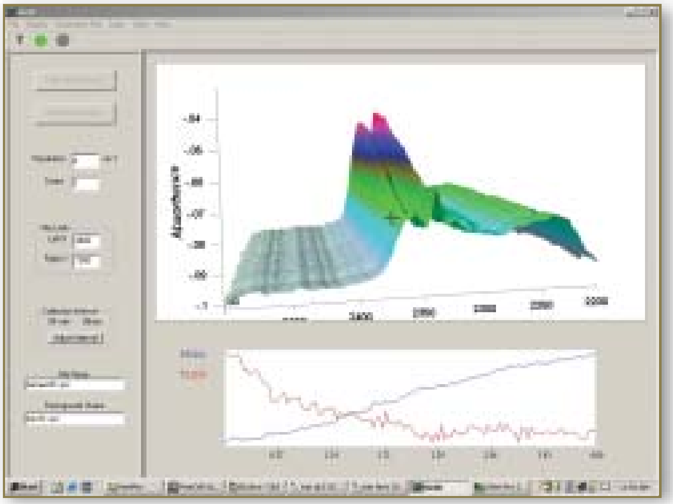
Experimental Conditions

The reaction was carried out in a stirred 1-L glass reactor. A ReactionView™ reaction monitoring system, running VizIR™ and fitted with a ZnS ATR crystal was used to track the reaction; an air background was used and spectra were collected every 2 min. at 4 cm^{-1} resolution from 900–5000 cm^{-1}. Glucose (50 g) was dissolved in water (500 mL); the first mid-IR spectrum was collected, then the solution was inoculated with a yeast starter. The fermentation was carried out at 24°C for three hours.

Results

Two spectroscopic ranges are of interest in sugar fermentations. The area from 1000–1200 cm^{-1} exhibits characteristic absorbances from the sugars and alcohols, in this case glucose and ethanol. Typically, these spectra are superimposed and cannot be quantitatively analyzed by simple peak-height or peak-area measurements, although crude trend lines can be based on intensity changes in this area of the spectra. For quantitative analysis, chemometric methods are needed.

The second range of interest is between 2300 and 2400 cm^{-1}, where the CO_2 spectrum is observed. As the fermentation proceeds, CO_2 initially appears in its dissolved form, characterized by a singlet at 2342 cm^{-1}. The gaseous CO_2 inside nucleating bubbles is also detected in the ATR experiment, as an asymmetrical doublet at 2361 and 2326 cm^{-1}. The resulting pseudo-triplet that evolves during the course of the reaction is shown in the upper window in Figure 1 (a screen shot of the realtime VizIR™ display). This type of spectroscopic feature lends itself to peak-fitting techniques for the calculation of peak areas, and the resulting peak model can be used in real time to calculate trend lines for the individual features, as shown in the lower window in Figure 1 for the peak at 2342 cm^{-1}. In a final step, the mid-IR spectrum of dissolved CO_2 can be conveniently calibrated against known concentrations in buffered sodium bicarbonate solutions. Thus, a direct, quantitative measurement of dissolved CO_2 has been developed that can be used inside a wide variety of reactors.



Conclusions

In-situ mid-IR spectroscopy opens a spectroscopic window into fermentation reactions. The ATR probe works well in the typically cloudy and effervescent media found in fermenters and the real-time integration of methods such as peak fitting and chemometric calibrations into the software interface promises to turn the method into a powerful reaction-profiling tool.

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