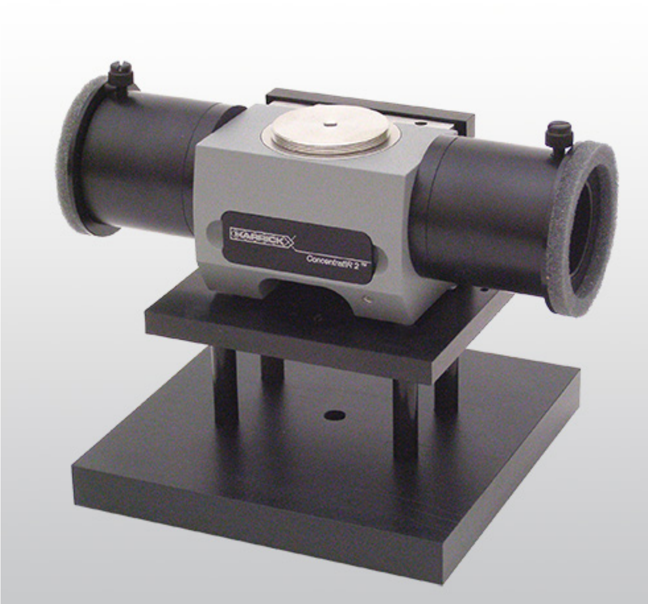

Figure 1. The ConcentratIR2™ multiple reflection ATR accessory.

Analysis of Yeast Fermentation Using the ConcentratIR2™

INTRODUCTION

Attenuated total reflectance (ATR) infrared spectroscopy is a powerful spectroscopic technique primarily due to the use of very small sample sizes (on the order of μL). The short effective pathlength allows for the study of species in aqueous solutions. One potential application of ATR is the study of fermentation.

In this application note, the conversion of sucrose into ethanol by various strains of yeast is observed over time using the Harrick ConcentratIR2™ multiple-reflection diamond ATR accessory. Both sucrose and ethanol exhibit strong absorption bands in the infrared and thus ATR can be used to track the progress of the fermentation by observing the change in relative intensity of the bands.

EXPERIMENTAL

Four types of Lavlin yeast were used, Bourgovin RC 212 Dry Wine Yeast, EC-1118 Dry Wine Champagne Yeast, K1V-1116 Yeast, and ICV D47 Yeast. For each type of yeast, 1.5 g of sugar was dissolved in 50 mL tap water and then 1.5 g of yeast was stirred in. The mixtures were stirred approximately every 15 minutes and were covered with plastic when not stirred to prevent significant evaporation. All mixtures remained at room temperature, in the range of 22-25 °C, during the course of this study.

Infrared spectra were collected on an FT-IR spectrometer equipped with the Harrick ConcentratIR2™ accessory with a diamond ATR crystal. A background was collected with the accessory in the sample compartment and no sample on the crystal. Spectra were the result of 128 averaged scans at 4 cm^{-1} resolution collected with a DTGS. The gain was set to 8 and the aperture was set to 100 (fully open). The spectrometer and accessory were purged with filtered air (H_2O and CO_2 removed) provided by a Parker Balston Model 75-62 FT-IR Purge Gas generator. The spectrum of pure water, collected

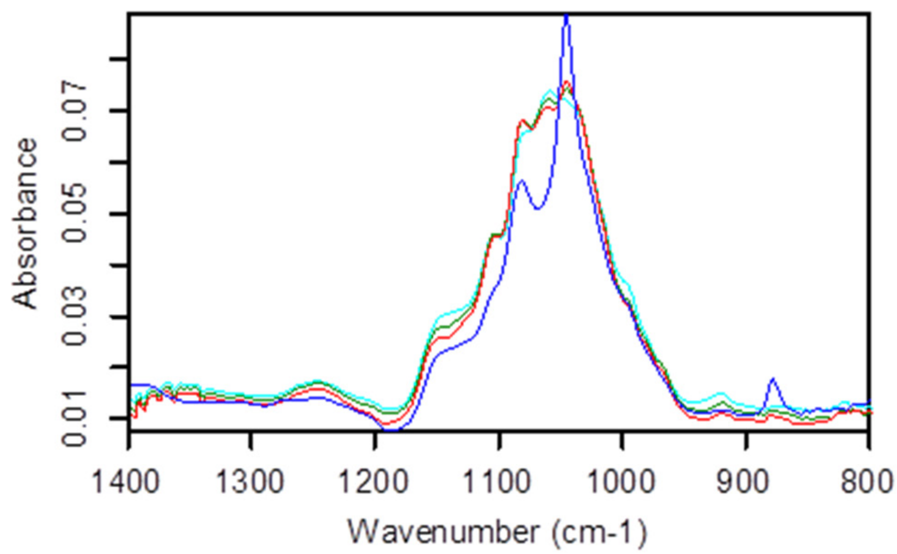

Figure 2. Infrared spectra of D47 yeast fermentation. Teal: 1h, Green: 2h, Red: 3h, Blue: 24h

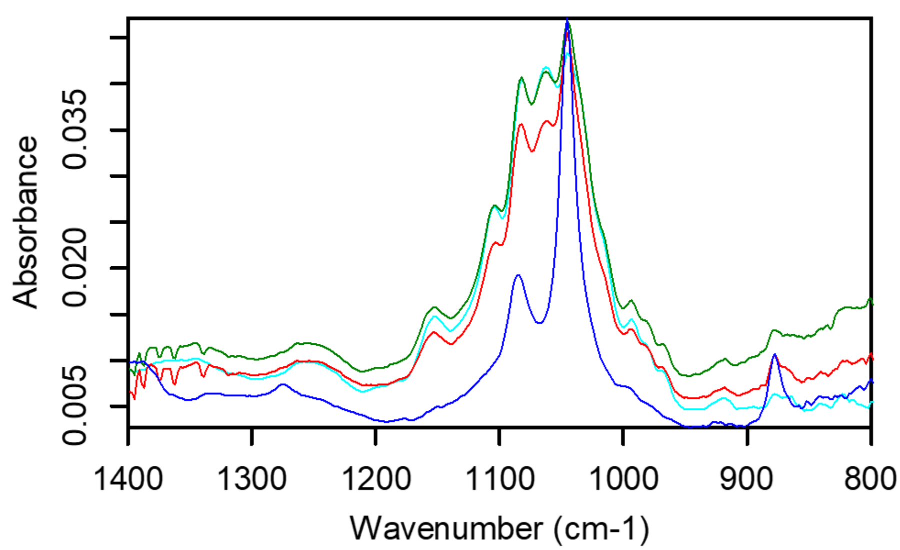

Figure 3. Infrared spectra of EC1118 yeast fermentation. Teal: 1h, Green: 2h, Red: 3h, Blue: 24h

under the same conditions, was subtracted from each sample spectrum.

RESULTS AND DISCUSSION

Four sets of spectra are shown in Figures 2-5, one for each yeast strain. Each set contain spectra from the first 3 hours of fermentation and a spectrum taken approximately 24 hours after fermentation began. The set of overlapping bands from approximately 1200-950 cm^{-1} correspond to sucrose. Ethanol also has two bands in the same range, which are initially hard to see due to the sucrose bands but emerge as the sugar concentration decreases. Looking at these peaks, it is clear that the EC1118 yeast is the fastest fermenter, as expected since it is rated as a fastest fermenter with the lowest recommended temperature range[1] of those investigated. The RC 212 appears to be the next fastest, with the K1V-1116 and ICV-D47 yeast acting more slowly. These results demonstrate that it is possible to observe the fermentation process with multiple reflection diamond ATR using the ConcentratIR2. A more detailed analysis could be performed using partial-least squares (PLS) to statistically estimate the concentrations of sucrose and ethanol, as has been done elsewhere[2,3].

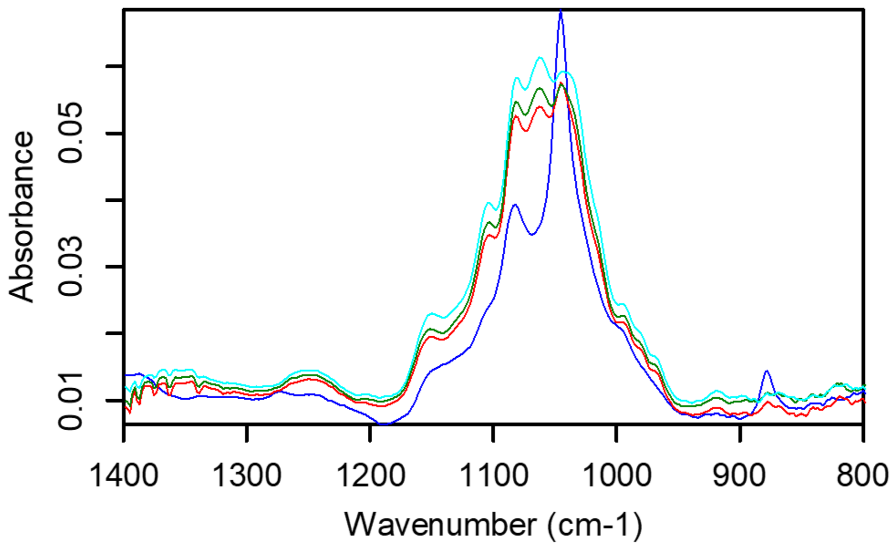

Figure 2. Infrared spectra of D47 yeast fermentation. Teal: 1h, Green: 2h, Red: 3h, Blue: 24h

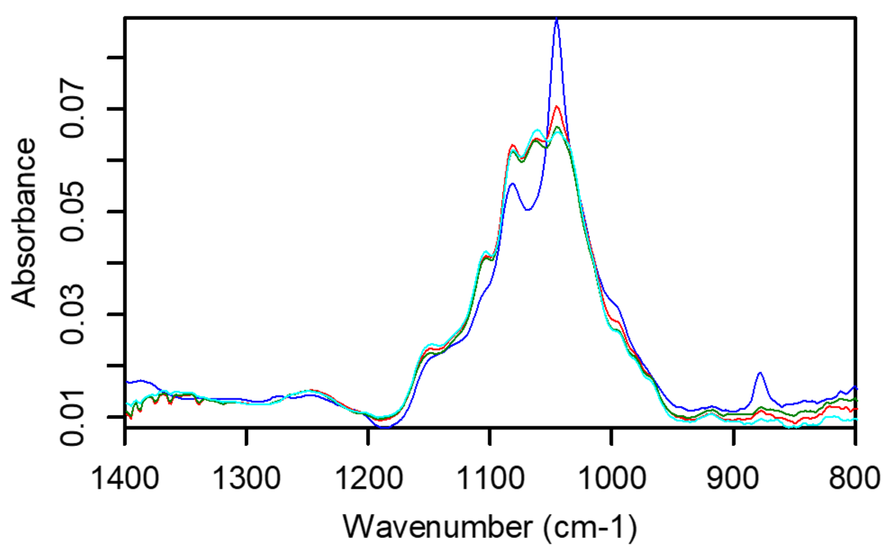

Figure 5. Infrared spectra of RC yeast fermentation. Teal: 1h, Green: 2h, Red: 3h, Blue: 24h

CONCLUSIONS

ATR spectroscopy is a powerful method that can be used to analyze many different samples, particularly aqueous samples. Yeast fermentation in an aqueous sucrose solution was observed using the Harrick ConcentratIR2™ ATR accessory. It was immediately possible to see relative rates between the different yeasts, but further analysis is possible to obtain more quantitative data or to derive reaction rates.

REFERENCES

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2. Di Egidio, V.; Sinelli, N.; Giovanelli, G.; Moles, A.; Casiraghi, E. *Eur. Food Res. Technol* **2010**, 230, 947-955.
3. Mazarevica, G.; Diewok, J.; Baena, J.R.; Rosenberg, E.; Lendl, B. *Applied Spectroscopy*, **2004**, 58, 804-810.