METABOLIC MICROSCOPY: NOVEL APPROACHES TO HIGH THROUGHPUT SCREENING IN STRAIN DEVELOPMENT AND FERMENTATION ANALYSIS USING DIFFUSE REFLECTANCE FT-IR AND DISPERSIVE RAMAN SPECTROSCOPY WITH CHEMOMETRICS

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ABSTRACT

Analysis of metabolites and biotransformation products in High Throughput Screening (HTS) and fermentations can be restricted by relatively slow assay methods, such as HPLC. Diffuse reflectance absorbance FT-IR spectroscopy and Raman spectroscopy provide rapid, automated and quantitative approaches which can be used non-invasively on unprocessed complex fermentation samples. We demonstrate how these spectroscopic techniques, in combination with chemometrics, can be used to quantify the concentration of metabolites in microbial fermentations. Supervised and unsupervised multivariate calibration methods were used to analyse the FTIR spectra of culture supernatants from *Streptomyces citricolor* mutants overproducing carbocyclic nucleosides. The utility of dispersive Raman spectroscopy for quantifying biotransformation products on-line in fermentations was demonstrated with the non-invasive analysis of the microbial conversion of glucose to ethanol.

INTRODUCTION

Diffuse reflectance infrared spectroscopy

We have shown previously that DRIFT (Diffuse Reflectance Infrared Fourier Transform) spectroscopy can be used for the accurate determination of ampicillin concentration in a background of *Escherichia coli* and *Staphylococcus aureus* cells (Winson *et al.*, 1997). To show how DRIFT can be used to measure fermentation products in a strain development programme, we investigated the production of aristeromycin and neplanocin A, carbocyclic nucleosides produced by *Streptomyces citricolor*. DRIFT analysis was performed using a Bruker IFS28 FTIR spectrometer equipped with a diffuse-reflectance TLC attachment and a liquid N_2-cooled MCT detector. Unprocessed culture supernatants (5µl; four replicates) sampled after 6 days incubation were dried in the wells of an aluminium plate mounted on a motorised stage and infrared spectra were collected in the wavenumber range 4000 - 600 cm^{-1} (16 co-adds). Spectral data were converted to ASCII format and analysed using partial least squares regression to determine the nucleoside concentrations (Figure 1).

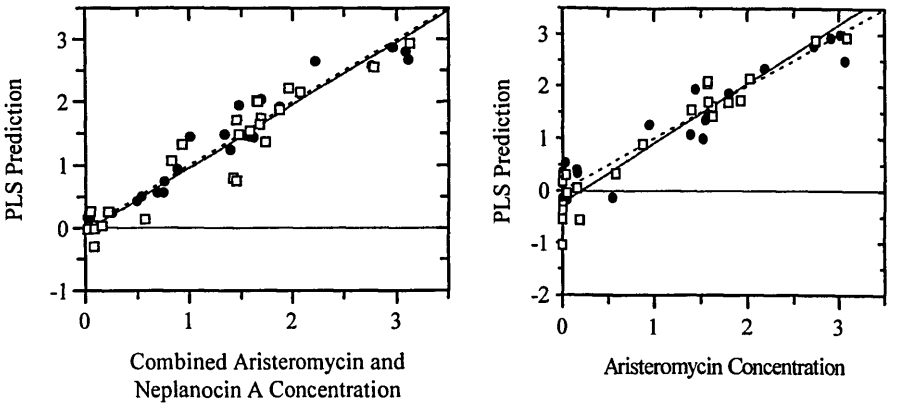

FIGURE 1. Partial least squares (PLS) prediction of combined aristeromycin and neplanocin, and aristeromycin concentrations ($g.l^{-1}$) in *S. citricolor* mutant culture supernatants measured by IR. Nucleoside concentrations were determined by HPLC.

Dispersive Raman spectroscopy

On-line analysis of anaerobic fermentation of glucose to ethanol by *Saccharomyces cereviseae* was monitored using the Renishaw dispersive Raman spectrometer with a low power (30mW) near-infrared 780nm diode laser giving 3mW power at the sampling point. The fermentation was performed under anaerobic conditions in 90ml of minimal medium (pH4.5) (containing 9g D-glucose, 0.05% yeast extract and 0.05% peptone) and the Raman dispersive spectra recorded at intervals (Figure 2).

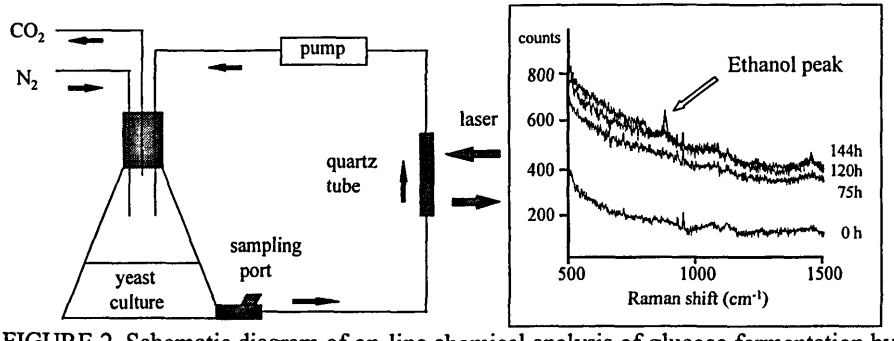

FIGURE 2. Schematic diagram of on-line chemical analysis of glucose fermentation by yeast using dispersive Raman spectroscopy.

REFERENCE

Winson, M.K., Goodacre, R., Timmins, É.M, Jones, A., Alsberg, B.K., Woodward, A.M., Rowland, J.J. and Kell, D.B. (1997) Diffuse reflectance absorbance spectroscopy taking in chemometrics (DRASTIC). A hyperspectral approach to rapid screening for metabolite overproduction *Analytica Chimica Acta* **348:** 273-282.

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