FACTORS INFLUENCING ON-LINE ANALYSIS OF FERMENTATION ALCOHOLS BY CAPILLARY INLET MASS SPECTROMETRY

J.P. Camelbeeck, D.M. Comberbach*, M. Orval+, J.O. Pêtre and P. Roelants

SmithKline Beecham Biologicals + Institut Supérieur Industriel Liégeois (I.S.I.L.)
Rue de l'Institut 89 Quai Glosener 6
B-1330 Rixensart, Belgium B-4000 Liège, Belgium

SUMMARY

The two most important factors for rapid, precise analysis of fermentation alcohols by on-line mass spectrometry were the "real" response time of the mass spectrometer and the adsorptive capacity of the tube transporting the off-gas. At this scale (20 l), the bulk liquid mixing time, and the rate of attainment of vapour-liquid equilibrium (VLE) in the fermenter were of lesser importance. No departure from fermenter VLE was observed under extremes of agitation speed, aeration rate, low liquid volume and pH. The total system response time (t<sub>90%</sub>) to a pulse of ethanol injected directly into the fermenter liquid was about 6 minutes.

INTRODUCTION

Previous work has demonstrated the use of capillary inlet mass spectrometry to follow alcohol concentration in fermentation broths indirectly via the partial pressure in the off-gas (Camelbeeck et al, 1989). The success of this technique depended largely on the controlled physical environment found within the fermenter vessel (temperature, pressure). This favoured the rapid attainment of vapour-liquid equilibrium (VLE). However, there are other factors which reduce the availability of alcohol molecules for detection.

Early pulse-response experiments using a stainless steel capillary inlet at ambient temperature to monitor alcohol partial pressures in off-gas gave slow response times and memory effects resulting from molecular adsorption/desorption from/to the gas stream (Josland, 1987). The capillary inlet was subsequently modified to incorporate a heater, and to reduce the contact of sample gas with cold metal.

A similar problem occurred in the transportation of fermenter off-gas to the rotary valve of the mass spectrometer. Other workers found a response time (t<sub>90%</sub>) of > 24 h when transporting propanol vapour through stainless steel tube maintained at 34° C (Bobatka et al, 1983). Experiments at SB Biologicals with different types of tube of equal length and internal diameter using alcohol vapour at room temperature gave similar results. The following trend was obtained:

high molecular adsorption > SS > PVC > PVDF > PTFE > low molecular adsorption

As noted by other workers (Heinzle et al, 1987), PTFE adsorbed alcohol molecules the least, and was therefore used in this study.

These two modifications alone were enough to improve total system response times from hours to minutes, making this technique a practicable replacement for traditional off-line alcohol analysis by either gas chromatography or enzyme test kits. The technique has been in use by the microbiology and fermentation research and development department at SB Biologicals for the last two years, analysing the concentrations of ethanol (EtOH) and methanol (MeOH) on-line, in yeast fermentations. During that time, several aspects of the technique have been studied (Orval, M., 1989) with a view to improving the total system response time, and eventually, to perform feedback process control.
THEORY

The total system response time (t_s) for the detection of an alcohol in the fermenter liquid is the sum of several individual response times, the lengths of which are influenced by rates of attainment of equilibrium:

\[ t_s = t_{BM} + t_{VLE} + t_{ME} + t_{MS} \]

where:

- \( t_{BM} \) is the time required after the appearance of alcohol in the liquid for bulk mixing to take place. It depends largely on agitation speed and fermenter geometry.
- \( t_{VLE} \) is the time required for VLE to be established in the off-gas. It depends on temperature, pressure, aeration rate and relative volumes of liquid and headspace.
- \( t_{ME} \) is the time for molecular equilibrium to be established in the gas transport tube. It depends on the adsorptive properties of the tube (a function of length, superficial gas velocity, temperature and internal surface characteristics).
- \( t_{MS} \) is the time required for the mass spectrometer to provide an on-screen analysis of alcohol concentration in the off-gas from time of entry into the rotary valve. It depends on the individual characteristics of the mass spectrometer (capillary inlet design, sensitivity of response, etc.) and the practical analysis method (number of additional components to be determined - N_2, O_2, Ar, CO_2 + alcohol 1 + alcohol 2, settling time between analyses, etc.).

A value for \( t_{BM} \) may be estimated from the literature (Van't Riet and Tramper, 1991) for typical conditions found in a laboratory fermenter (i.e. two-phase air-water mixture with flat-blade impellers creating turbulence with \( Re > 10^4 \)). Typical values for \( t_{BM} \) under the above conditions are 10-20 s.

The \( t_{VLE} \) is the most important value of all the individual response times since the technique depends upon gas-phase components being in equilibrium at all times with those in the liquid. For this reason, several factors likely to influence the rate of attainment of equilibrium (temperature, aeration rate, liquid volume, etc.) have been studied.

The \( t_{ME} \) is the second most important variable after \( t_{VLE} \) since it could, in the worst case, increase the total system response time to a level where the total system response time is too slow for realistic on-line analysis, and in the best case, represent only a fraction of \( t_s \). Thus the manner in which the off-gas is transported from the fermenter to the mass spectrometer is of utmost importance to the success of this technique. For this reason, one factor likely to influence \( t_{ME} \) has been studied (tube temperature).

The \( t_{MS} \) value for individual components is sometimes quoted in sales brochures by manufacturers of mass spectrometers, and such values for lower alcohols are surprisingly low (90% < 5 s). However, these values are often obtained by measuring the current from one specific ion (e.g. for MeOH or EtOH, m/e = 31), and are performed by placing the capillary inlet directly into the headspace of a bottle containing pure alcohol. Response times measured in this way are not representative of the way the mass spectrometer is used in practice. Typical workload for a fermentation mass spectrometer would be the analysis of six components (N_2, O_2, Ar, CO_2, EtOH, MeOH) with a total scan time per fermenter of 25 s. The data-handling software would then report the complete analysis before the rotary valve selects the next fermenter in the programmed sequence. The \( t_{MS} \) value is therefore very specific to the mass spectrometer/sample handling/data analysis system, and therefore cannot be changed without modification to the mass spectrometer and peripherals. The "working" \( t_{MS} \) value is therefore likely to be in the order of minutes rather than seconds.