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AN IMPROVED CHROMATOGRAPHIC PROCEDURE FOR THE RAPID DETERMINATION OF VOLATILE SOLVENTS VIA THE HEADSPACE GAS

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SUMMARY

An improved chromatographic technique was developed to monitor the acetone/butanol fermentation 'on-line'. Reduction in column length and diameter, use of a compromise oven temperature for multicomponent systems and smaller headspace gas aliquots permitted the completion of chromatograms within 3.5 minutes. Transient solvent concentrations were sensed within 2.5 minutes, and the chromatographic response was linear to 110 g/L ethanol and 20 g/L acetone and butanol concentrations.

INTRODUCTION

Only two analytical procedures are specific for the continuous monitoring of individual components or mixtures arising from the headspace gas of traditional solvent fermentation processes. These are mass spectrometry (MS) and gas chromatography (GC) (Comberbach and Bu'Lock, 1983). Mass spectrometry can be used to monitor fermentation processes involving a single solvent, ethanol (Pungor et al, 1980), or multiple solvents, ethanol, acetone and butanol (Doerner et al, 1982). However, MS is susceptible to membrane inlet associated problems (slow response times, nonlinear calibration and membrane memory effects), and does require sophisticated software to interpret the results.

Recent work has shown that headspace gas chromatography (HSGC) can be used to monitor the progress of both batch and continuous ethanol fermentations by repeated injection of HS gas aliquots (Comberbach, 1981). Gas injection, column back-flushing and peak area integration were facilitated by a computer-controlled gas chromatograph which gave a complete analytical report every six minutes.

The following work describes the adaptation and optimisation of the HSGC procedure for the continuous determination of volatiles from the acetone/butanol fermentation.

MATERIALS AND METHODS

Fermenter - an external loop gas-lift tower fermenter (GLTF) with working and headspace volumes of 8.9 and 1.55 litres respectively, was used for both calibration and fermentation (Figure 1). The contents were mixed by the injection of filtered (F_1) CO_2 via a pressure regulator (PR) and gauge (PG). The temperature was held constant (T_1) by a thermistor-controlled (T_3)

heating circuit. A major portion of the efflux gas was vented to atmosphere via a water-cooled condenser (C), a precalibrated rotameter (R) and finally an exit filter (F_3). The remaining fraction of headspace gas was transported through a stainless steel heated gas line (HGL) to the gas chromatograph via stainless steel lagged tubing (LT).

Gas Chromatograph - the GC (Perkin-Elmer, Sigma 2000) was located adjacent to the fermenter, and was equipped with a heated, ten port, two position, automatic gas sampling valve (AGSV) which enabled samples of headspace gas to be injected onto the column (Figure 1). Headspace gas was continuously removed via the heated sample loop (SL, 0.1 ml) by vacuum pump (P_1), at a constant rate, adjusted by the regulator (VR) with reference to the vacuum gauge (VG). The AGSV was activated electropneumatically from the Timed Events program in the GC method. The method was stored in the data station (Perkin-Elmer, Sigma 15), the latter providing peak monitoring, area integration and report printout after each injection.

Chromatographic Conditions - A 1 m long nickel column (3.175 mm o.d., 2.175 mm i.d.) was used with Porapak Q (100-120 mesh) as the packing material. To minimize condensation, the gas line and the sample loop were heated to 90°C during calibration and to 120°C during actual fermentations. The detector (FID) was maintained at 250°C and the carrier gas (oxygen-free N_2) was supplied at 30 mL/min.

For external standard analysis of individual solvents, optimal oven temperatures differed; 180°C for ethanol, 210°C for acetone and 230°C for butanol. As a compromise, 200°C was selected when all three solvents occurred in mixtures. For conventional manual internal standard liquid analysis of both samples, isothermal temperatures of 230°C and 200°C were used for both injector and oven respectively.

Organism - a strain of Clostridium acetobutylicum (ATCC 824) was used throughout this work.

Medium - inocula of Cl. acetobutylicum were grown anaerobically in a synthetic medium of the following composition (for 1 litre deionised water): glucose monohydrate, 22 g; yeast extract, 5 g; (NH_4) $COOCH_3$, 2 g; $FeSO_4$ $7H_2O$, 0.01 g; $MnSO_4$ $4H_2O$, 0.01 g; $MgSC_4$ $7H_2O$, 0.1 g; KH_2PO_4, 0.75 g; K_2HPO_4, 0.75 g. The pH was adjusted to 5.5 with 4M HCl, and sterilized for 15 minutes at 121°C. The concentrations of medium components for the batch fermentation were the same as above excepting glucose monohydrate, which was raised to 40 g/L. The glucose solution was sterilised separately before being added to the fermenter.

Culture Conditions and Analytical Methods - a 5% (v/v) inoculum of Cl. acetobutylicum was prepared using the maintenance medium and incubated at 30°C for 24 hours under an atmosphere of CO_2. The inoculum was transferred to the fermenter containing presterilised medium, maintained at 30 ± 0.25°C and sparged with CO_2 at 0.075 vvm. The initial pH was adjusted to 5.5, and silicone antifoam was added when required.

Headspace gas injections were performed manually or automatically when required (every 2.5 minutes for individual solvent calibration, every 15 minutes for the fermentation) by rotation of the AGSV from position A (SL fill) to position B (SL inject).

Aliquots (50 ml) of fermentation broth were periodically removed and frozen at -25°C for the subsequent analyses of bacterium, glucose, ethanol, acetone and butanol. Pre-thawed samples were centrifuged (1500 g, 5 minutes)

and the resulting pellet was washed and dried to constant weight for the determination of bacterial cell concentration. Glucose concentration was determined from the supernatant using a commercially available test kit (Boehringer, Ltd.), and ethanol, acetone and butanol were determined 'off-line' by conventional manual liquid injection gas chromatography (Sigma 3, Perkin-Elmer Ltd.) using n-propanol as the internal standard.

Chromatograph Calibration - the GC was calibrated using pure (analytical grade) solutions of acetone, ethanol and butanol in deionized water from 2-25 g/L in the fermenter at 30 ± 0.25°C. The CO_2 input was adjusted to 0.075 vvm. A headspace gas throughput of 150 mL/min was maintained, which enabled gas transfer from fermenter to GC to occur within 1.5 seconds. This was done to investigate the linearity of detector signal response to solvent concentration, and to calculate the absolute response factor F_i. Each automatic analysis was performed at least 5 times to determine the mean, the standard deviation and the percent standard deviation.

Further details concerning fermenter design, apparatus layout, chromatograph operation, culture conditions and analytical methods are described elsewhere (Comberbach et al, 1984).

RESULTS AND DISCUSSION

A typical headspace gas chromatogram from a 5-5-5 g/L aqueous mixture of acetone, ethanol and butanol can be seen in Figure 2. Previous workers (Comberbach and Bu'Lock, 1982) using a similar HSGC technique to analyse fermentation ethanol required a carrier gas flowrate of 40 mL/min to elute ethanol at 4.3 minutes, a result of the longer (2 m) column in use at the time. The narrow bore nickel column provided a high pressure drop, which removed the necessity for a downstream restrictor tube (the incorporation of which was previously required to prevent the detector flame from being extinguished each time the AGSV was rotated).

Headspace signal to liquid concentration calibration curves for individual solutions of acetone and butanol were linear at least to 20 and 25 g/L respectively (higher concentrations were not expected in the acetone/ethanol/butanol fermentation), and the calibration curve for ethanol only became non-linear at liquid ethanol concentrations exceeding 110 g/L (Figure 3). This represents a 37% improvement in linearity over results previously reported (Comberbach, 1983), due at least in part to a 90% reduction in SL volume (0.1 mL). Analytical precision tended to decrease when one component was analysed in the presence of 5-5 g/L aqueous solutions of the remaining two (Table 1), probably because of reduced peak integration accuracy at the compromise (200°C) oven temperature. Individual activity coefficients changed (as monitored by changes in F_i) when the concentration of the component of interest in the mixture was varied. γ_{EtOH} decreased by 3%, and the remaining two, γ_{Me_2CO} and γ_{BuOH} increased by 2.8% and 1.9% respectively. Despite these small variations, all components gave linear signal to concentration responses up to 20 g/L in aqueous mixtures.

Component response time was a function of several parameters including the degree of mixing in the bulk liquid and headspace volumes, the HS gas throughput rate, the distance between the fermenter and GC, and the component elution rate. Figure 4 shows the headspace gas response to a pulse of pure acetone (45 g) added to an aqueous acetone solution. Completion of the step change in concentration and a return to steady-state occurred within the 2.5 minute interval between successive injections.

Sample chromatograms from a batch fermentation of Cl. acetobutylicum show its progress from late lag phase (A), early and late exponential phases

(B and C respectively), to stationary phase (D) (Figure 5). Such chromatograms, whilst providing a quantitative analysis of the major identified fermentation products also give a visual indication and qualitative estimation of transitory components (eg. the unidentified peak appearing 1.42 minutes into B). Figures 6, 7 and 8 show the headspace/liquid calibration curves for acetone, ethanol and butanol respectively from the fermentation. Headspace signal to concentration ratios remained linear for all three solvents, although the absolute response factor, F_i for each component decreased (Table 2) as a result of the increase in activity coefficients caused by the dissolved salt concentrations within the medium (Comberbach and Bu'Lock, 1983). Given the complexity of the fermentation broth, one can assume that those salts initially present in solution were either not depleted significantly or were replaced by other similarly active components excreted into the medium during cell growth. Thus the ionic strength of the broth remained essentially constant.

CONCLUSIONS

The continous removal of fermenter headspace gas for direct GC injection is a simple, non-invasive technique for monitoring the progress of single or multiple solvent fermentations. The small injection volume coupled with the FID's inherently wide dynamic operating range ensured rapid, precise measurements at both high and low component concentrations. Despite its short length, the separating column permitted adequate component selectivity, which together with the degree of precision on peak area determination, make this uncommon fermentation monitoring method ideally suited for process control.

ACKNOWLEDGEMENTS

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REFERENCES

Comberbach, D. M., "Continuous Alcoholic Fermentation. A Study of Ethanol Production Using a Novel Gas-Lift Reactor", Ph.D. Thesis, University of Manchester, October, 1981.

Comberbach, D. M., and Bu'Lock, J. D., Chromatogr. Newslett., 10, (1), 19-23, 1982.

Comberbach, D. M., and Bu'Lock, J. D., Biotechnol. Bioeng., 25, 2503-2518, 1983.

Comberbach, D. M., Scharer, J. M., Moo-Young, M., Biotechnol. Bioeng., (in preparation), 1984.

Doerner, P., Lehmann, J., Piehl, H., Megnet, R., Biotech. Letts., 4, 557-562, 1982.

Pungor, E., Perley, C. R., Cooney, C. L., Weaver, J. C., Biotech. Letts., 2, 409-414, 1980.

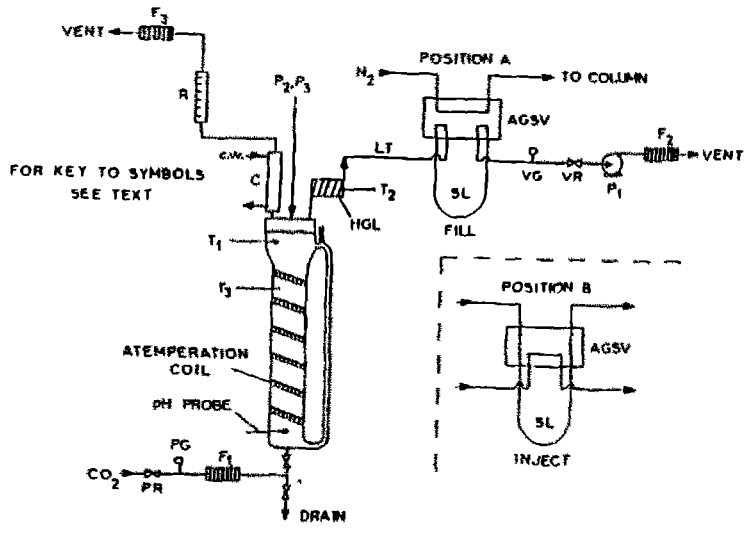

Fig. 1. Fermenter layout and piping flow diagram.

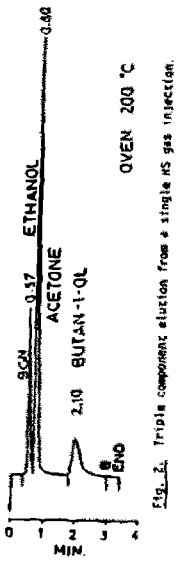

Fig. 2. Triple component elution from a single HS gas injection.

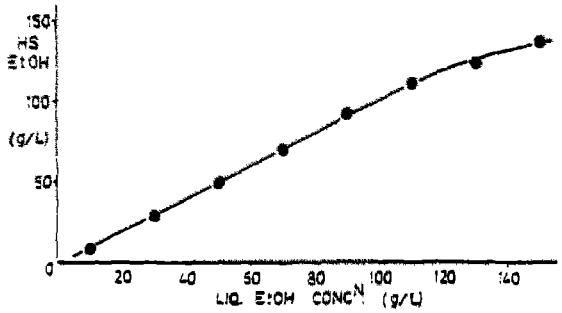

Fig. 3. Calibration of HS versus liquid ethanol concentration.

	Oven Temp.	Weighed Solvent Conc^n (g/L)	No. of Injections	F_1 for HS/L[3] Acetone = 1	Std. Dev.	RSD %
Individual Solutions						
Acetone	210°C	10	7	0.313	0.082	1.07 3
		15	5		3.346	2.50 4
Ethanol	180°C	10	5	0.127	0.011	1.37 3
		20	5		0.323	1.3 4
Butanol	220°C	10	5	0.048	0.379	0.55 3
		5	10		0.262	3.33 2
Mixtures						
Acetone	200°C	10	8	0.3126	0.106	1.07 3
		5	5		0.129	2.39 4
Ethanol	200°C	20	6	0.131	0.21	1.00 3
		5	6		0.185	3.74 4
Butanol	200°C	20	5	0.0468	0.398	1.91 3
		5	5		0.266	5.13 4

TABLE 1: Comparison of analytical precision between single and multicomponent elutions (3 = best RSD value, 4 = worst RSD value).

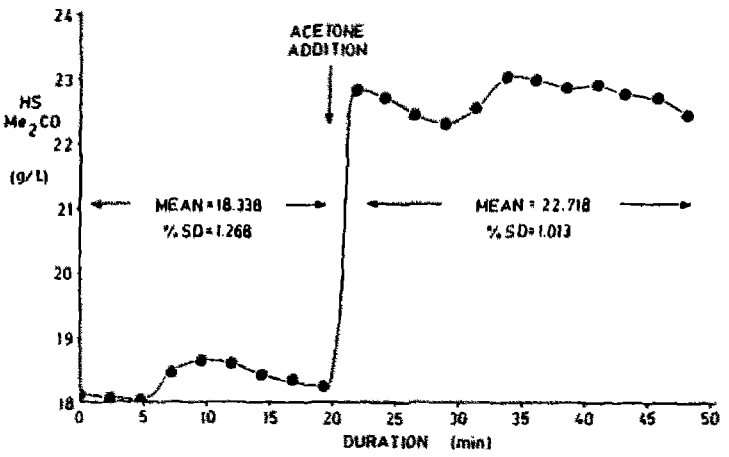

Fig. 4. HS gas response after pulse addition of pure acetone.

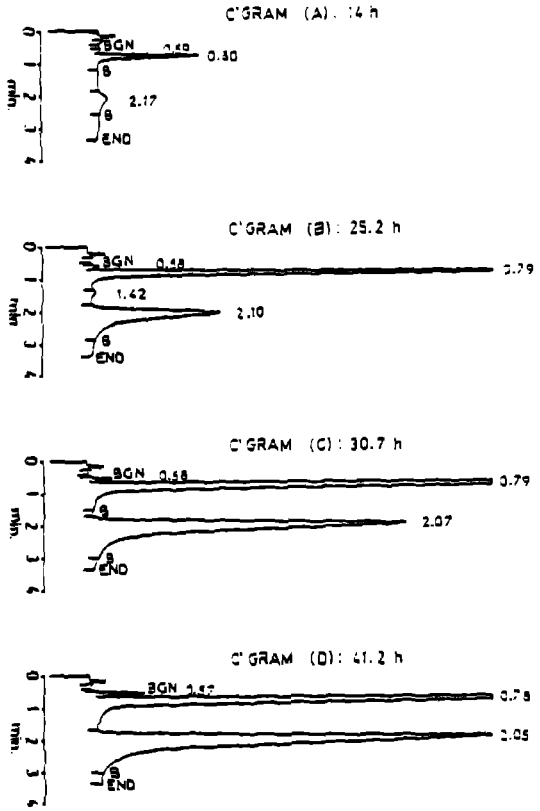

Fig. 5. HS gas chromatograms from the ABE fermentation.

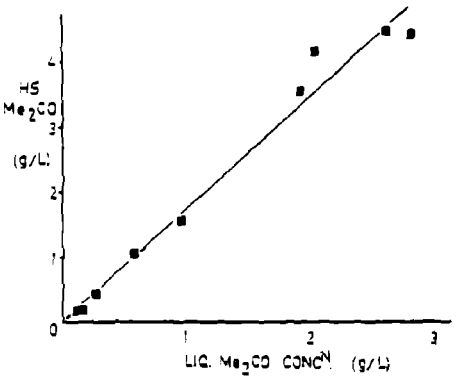

Fig. 6. Fermentation HS/LIQ acetone calibration curve.

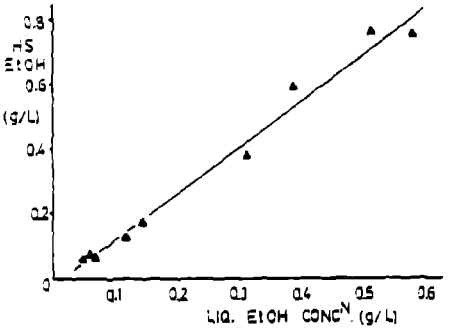

Fig. 7. Fermentation HS/LIQ ethanol calibration curve.

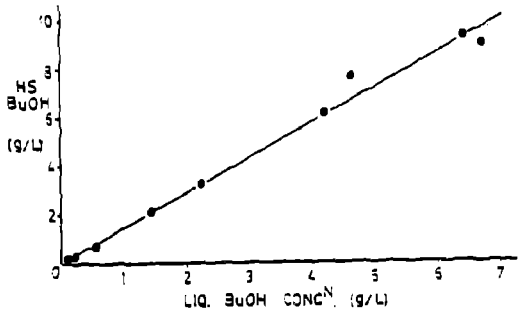

Fig. 8. Fermentation HS/LIQ butanol calibration curve.

	Acetone	Ethanol	Butanol
Figure	6	7	8
Coeff. of linear regression (r)	0.989	0.719	0.994
Absolute response factor (F_i)	0.3086	0.0892	0.0302
% Increase in activity coeff. (T_i)	29.9	49.2	36.3

TABLE 2: Quantitative evaluation of the fermentation results.