

Realtime measurement of volatile components in the bioreactor via proton transfer reaction mass spectrometry (PTR-MS) – an approach for advanced bioprocess monitoring

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Introduction

High sensitivity PTR-MS technology was implemented for on-line monitoring of volatile organic compounds (VOCs) in the off-gas of *E. coli* cultivations. An interface suitable for non-invasive, continuous on-line sampling of VOCs emanating from the bioprocess was developed. The whole system fully complied with the requirements for reproducibility and authenticity of the samples. Additional physiology relevant process information can be gained.

Setup

The PTR-MS bio system, the experimental setup for on-line VOC measurement (developed by Ionimed Analytik, Innsbruck, Austria in cooperation with the ACIB GmbH) consists of an inlet box and a PTR-MS.

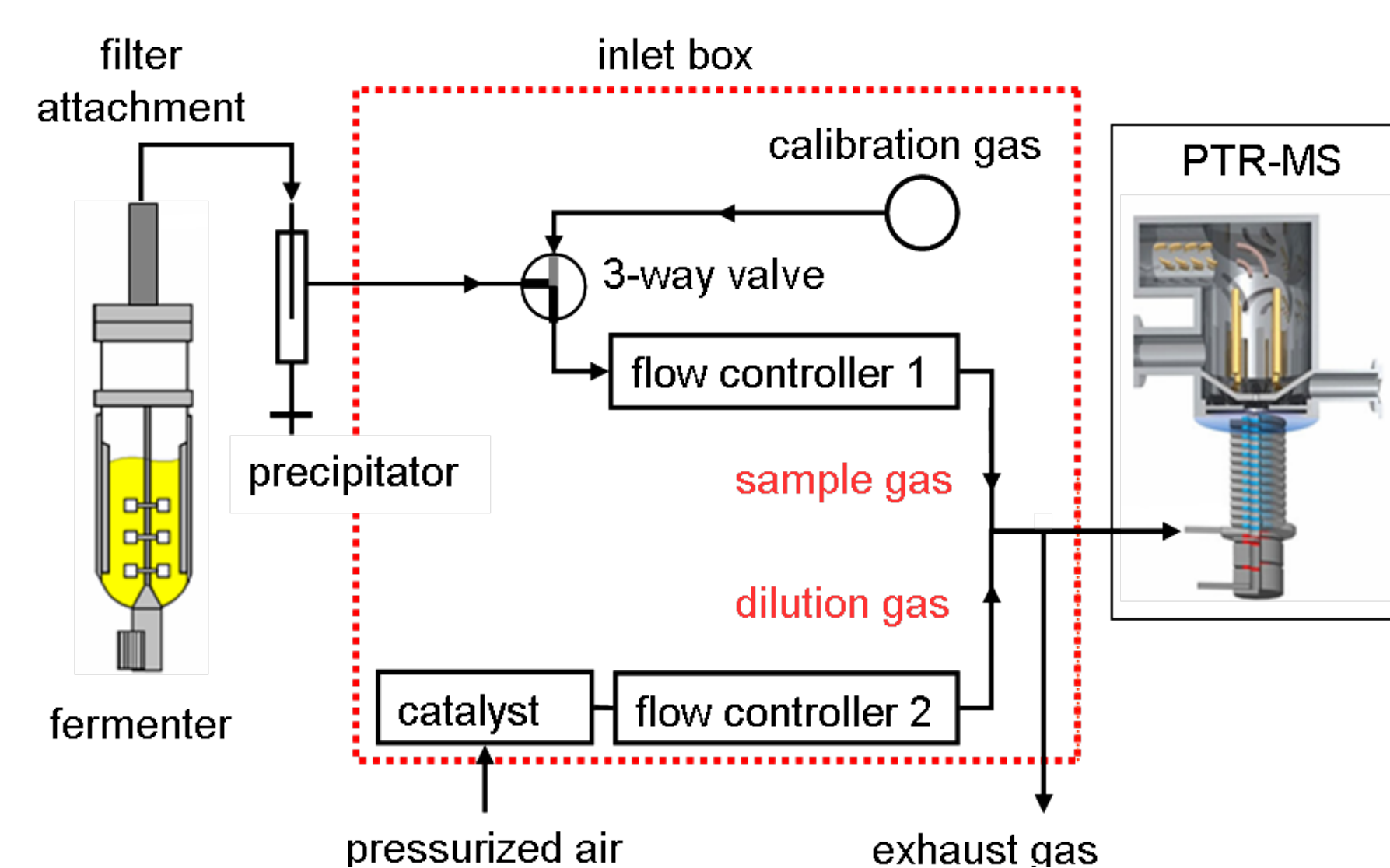


Figure 1: Simplified schematic of the setup. Fermenter off-gas is sampled and analyzed continuously. The inlet box allows for dilution and calibration and, if needed, multiplexing to analyze several fermenters.

Reproducibility

To demonstrate the proper functionality of the inlet system, three identical *E. coli* fermentations were conducted. The reproducibility of these experiments is shown by comparison of a set of process variables (Table 1, Figure 2).

	Pearson Correlation		
	cultivation 1 / 2	cultivation 1 / 3	cultivation 2 / 3
total CDM	0.998	0.996	0.999
spec. RP total	0.998	0.999	0.997
oxygen consumption	0.996	0.998	0.999
ex550 / em490	0.999	1.000	0.999
m/z 33	0.998	0.988	0.992
m/z 45	0.969	0.915	0.960
m/z 47	0.979	0.907	0.892
m/z 59	0.996	0.991	0.995
m/z 69	0.974	0.921	0.936

Table 1: Correlation coefficients for comparison of the similarity of on-line signals (PTR-MS) and process variables of three different cultivations.

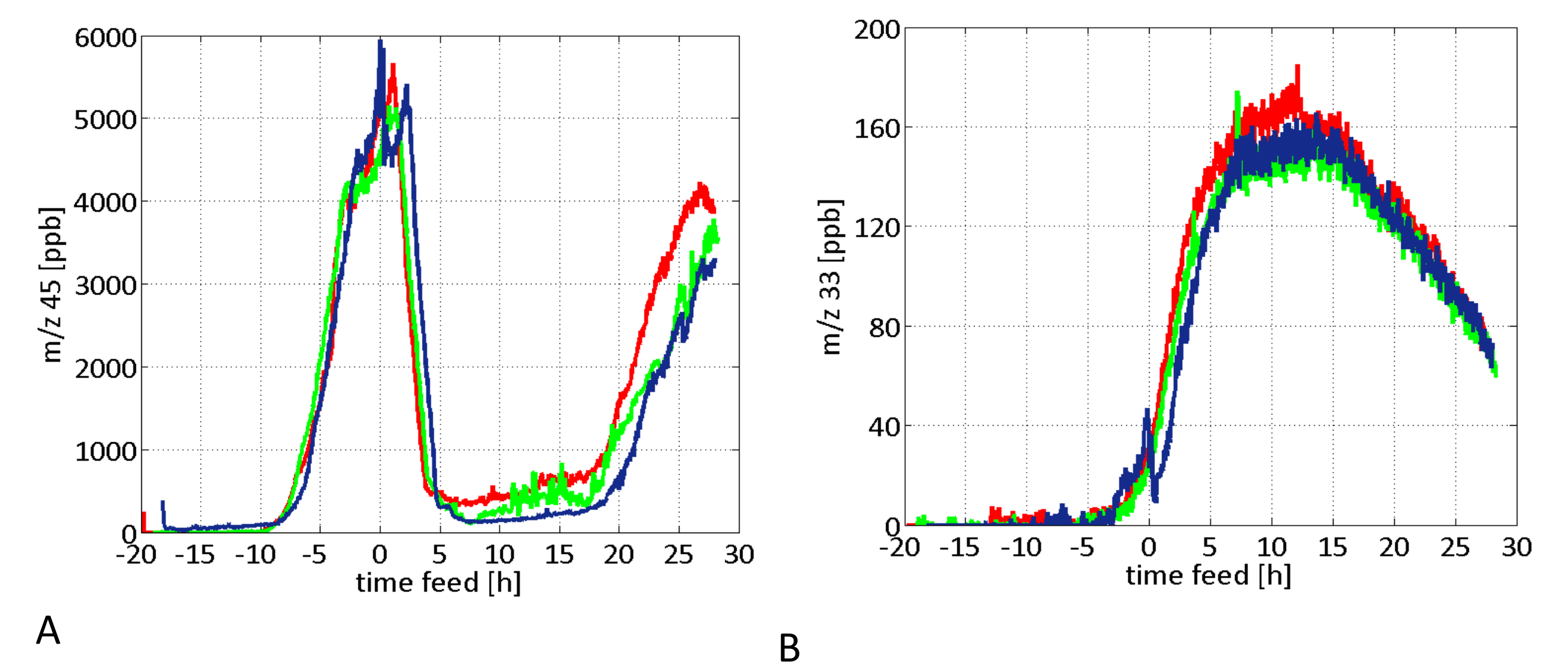


Figure 2: Typical PTR-MS signals from three recombinant *E. coli* fed-batch cultivations.

VOC-based process information

The volatiles acetaldehyde and ethanol detected with PTR-MS are metabolites involved in mixed acid fermentation. Both compounds accumulate during the batch phase as glucose is offered in excess during this phase. Re-use of these molecules in the early feed phase is clearly pictured in the decreasing PTR-MS signals but in the later stage of the process a switch to mixed acid fermentation metabolism is indicated again. Detailed off-line analysis shows that metabolic overload triggered by recombinant gene expression leads to reduced growth rates and consequently CDM deviates from the calculated course. This phenomenon coincides with the increase in acetaldehyde and ethanol in the fermenter off-gas and is directly visualized in the PTR-MS signals.

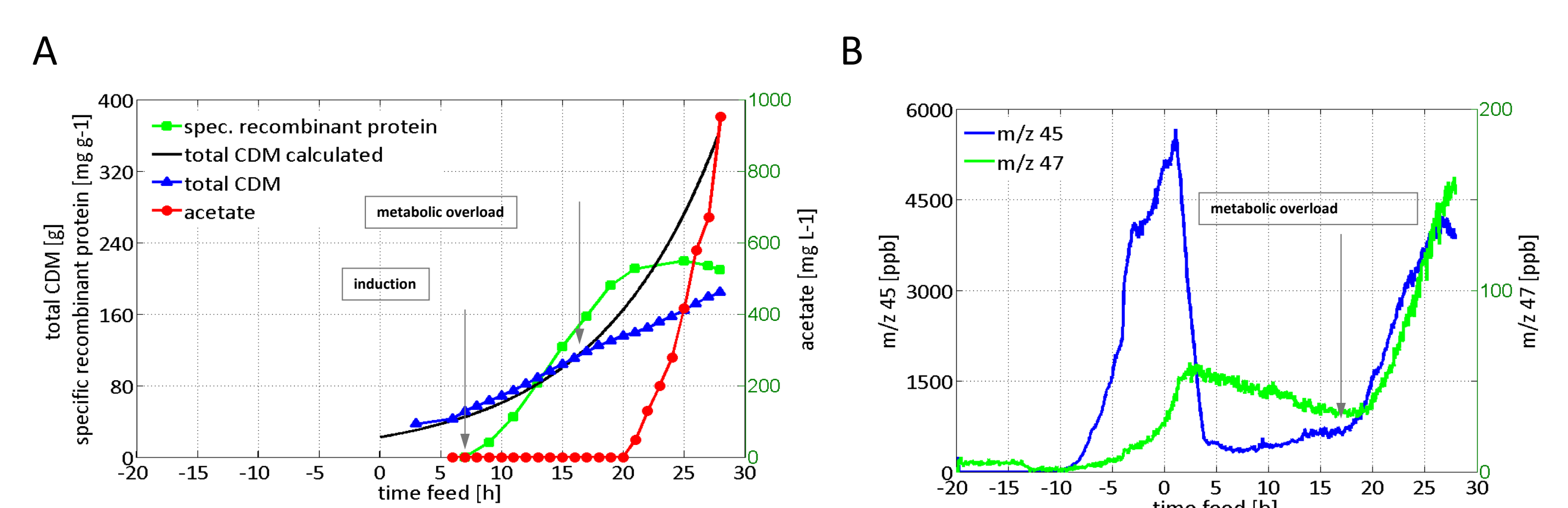


Figure 3: Recombinant *E. coli* fed-batch cultivation: (A) courses of calculated and total CDM, specific recombinant protein, and acetate; (B) courses of m/z 45 and 47 representing acetaldehyde and ethanol.

Summary

E. coli fermentations show characteristic trends of more than 20 volatiles in realtime. They are tightly linked to host cell metabolism. Additional process variables especially microarray data will give deeper insight into the origin of those VOCs and the involved metabolic reactions. On-line information on cell state enables the application of advanced process control regimes and accelerates the improvement of production process.

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