

Online Bacterial Headspace Sampling Setup for Medical Microbiology Diagnostics with Sensors

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Summary:

We introduce a headspace sampling setup for longitudinal monitoring of volatile emissions from microbial species that is compatible with various sensor methodologies. The setup was validated using mass spectrometry and chemoresistive sensors, showing reliable extraction with gas standards and *Escherichia coli* isolates over 24 h. Results show bacterial metabolic activity is mirrored in headspace volatile concentrations, with acetaldehyde serving as a key proliferation indicator. This proof-of-concept system enables continuous monitoring for rapid pathogen detection and antibiotic susceptibility evaluation.

Keywords: Volatilomics, Headspace sampling, Gas sensing, Microbiological sensing, Novel diagnostics.

Background, Motivation and Objective

Bacterial volatilome contains invaluable information on the bacterial metabolism that can be exploited to identify and characterize the organism. With the emergence of high-resolution analytical techniques like gas chromatography–mass spectrometer [1], ion mobility spectrometer [2], and secondary electrospray ionization spectrometer [3], quantitative analysis of headspace biomarkers has become feasible, and promising results were obtained for the identification of the bacteria [4] and the assessment of the antimicrobial resistance (AMR) risk [1]. Using sensitive and selective [5, 6] sensors, compact devices for volatilome analysis can be developed for rapid diagnostics. A crucial aspect of this analysis is the sampling method of the bacterial headspace. However, not only was the importance of sampling underestimated in the literature, but also the differences in sampling tools employed limited the direct comparability between studies. Proposed sorption methods are dependent on the sorptive material selection [7] and they can only perform integrated timepoint measurements. Employed commercial devices are not suitable for longitudinal monitoring [8], and custom-made solutions do not fully address the requirements for a reliable volatilome analysis [9]. The ideal method should be able to provide (i)

robust sampling control in terms of extracted volume and humidity for (ii) longitudinal monitoring with (iii) incubation conditions to account for the growth phase differences, (iv) online calibration for accurate volatilome quantification, and (v) controlled dilution of the sampled volume to accommodate dynamic range limitations. None of the proposed approaches provide such a standardized solution. Furthermore, this mismatch of volatilome sampling rendered analysis results incomparable, making the comprehensive understanding of the relationship between the volatilome and microbial metabolism an open challenge. We aim to close this critical gap between volatilome and microbial functions by proposing a standardized tool for controlled headspace volume extraction for both mass spectrometric and chemoresistive sensor measurements. The presentation starts with the essential factors for headspace sampling before introducing the setup with the corresponding design features to satisfy a representative sampling that can be used both for biomarker investigations and diagnostic sensing applications.

Description of the System

We defined the requirements for a reproducible volume extraction in bacterial volatilomics studies and designed a microbiological sample headspace sampling system to meet these

requirements (Figure 1). The setup is divided into two parts, the main line and the sampler line, to maintain a baseline flow for the analytical tool while precisely controlling the sampling, and to enable dilution of the extracted volume for dynamic range adjustments with limited humidity fluctuations. The flow rates and the flow humidity levels are controlled by the mass flow controllers; sample volume is extracted by a solenoid valve. The setup is enclosed and heated to 37 °C to deliver optimal incubation conditions to the microbiological samples. We have validated the approach with mass spectrometry using analyte gas standards. The sampling parameters were optimized for longitudinal analysis with target volatile organic compounds (VOCs). *In vitro* operation was validated with *Escherichia coli* isolates.

Results

Measurements with gas standards confirm reliable volume extraction, as measured down to 50 ppb. The dilution is shown to be an essential component of the volatilome analysis, for analytical tool operational range can be a limiting factor for higher concentrations. The sampling is validated using VOCs from various chemical classes, namely ketones, alcohols, aldehydes, and aromatics. The key aspects of headspace sampling are discussed through preliminary *Escherichia coli* measurements on MHB agar plates (Figure 2). We demonstrate that different classes of VOCs can be measured and bacterial proliferation can be monitored by quantifying the headspace VOC concentrations over the incubation period. The acetaldehyde emerged as the main indicator of bacterial proliferation in our conditions, as early as 4 h after inoculation.

Conclusion:

The versatile system is tailored to accurately sample the headspace of the bacterial culture while providing optimal incubation conditions for longitudinal monitoring. We validated our proof-of-concept device with demonstrative bacteria measurements; it enables standardized biomarker studies, as well as point-of-care testing by ensuring reproducible sampling.

Figures

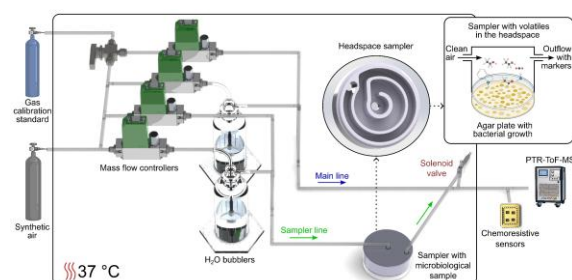


Fig. 1. 3D rendering of the headspace sampling setup

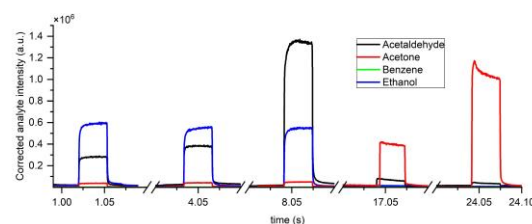


Fig. 2. Headspace concentrations of selected molecules from *Escherichia coli* inoculated on MHB agar.

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