Comparison of Real Time Off-Gas Analysis Equipment for DASGIP® Bioreactors: The DASGIP® GA4 Exhaust Analyzing Module vs. the Thermo-Scientific® (Prima Pro) Mass Spectrometer

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Introduction

Real time off-gas analysis is an important tool for gaining insights into a fermentation process by allowing the user to examine carbon evolution, oxygen utilization, and transfer during the fermentation. This information can be used to find changes in metabolism, spot potential problems, or trigger changes in the fermentation process.
We compared two technologies for off-gas analysis, namely a Prima Pro mass spectrometer (Thermo Fisher Scientific) and a DASGIP GA4 gas analyzer module (Eppendorf). The latter uses electrochemical and infrared sensors for the quantification of O_2 and CO_2, respectively.

Objectives

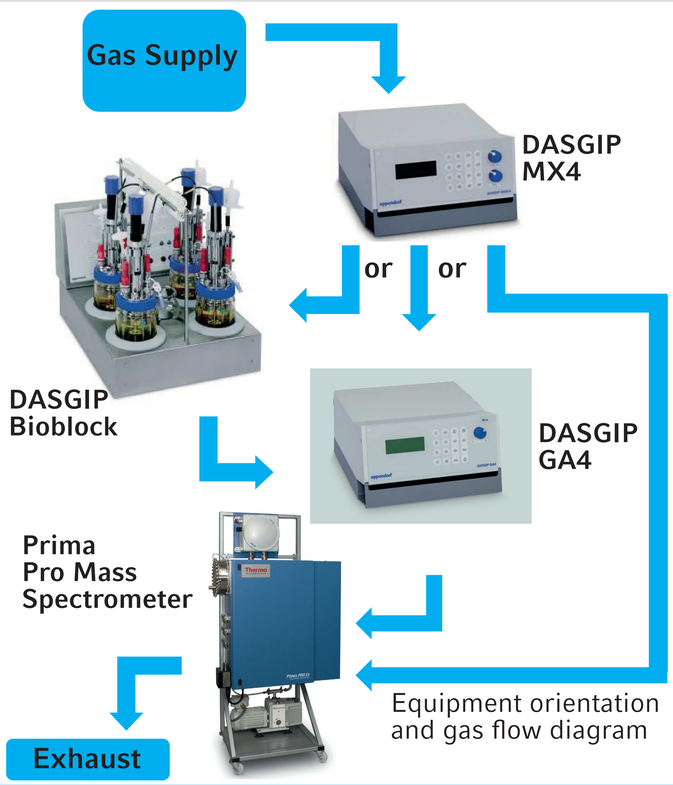
>Compare real time measuring capabilities of both the DASGIP GA4 and Prima Pro Mass Spectrometer
> Test both dry and humid (through a bioreactor) to observe impact of moisture on measurement readings
> Utilize both standard gasses and biological samples to compare the equipment
> Ensure two measuring devices in series would not interfere with one another

Materials and Methods

The DASGIP Parallel Bioreactor System (Eppendorf) was utilized for all experiments. Gas was supplied to the DASGIP MX gas mixing module which regulated air flow where it was either pushed through a BioBLU® 1f Single-Use Vessel (Eppendorf), or fed directly into the DASGIP GA4 off gas analyzer, which was followed in the chain by the Prima Pro mass spectrometer.

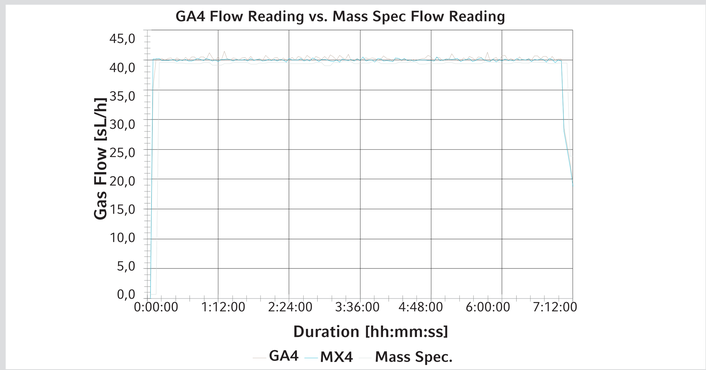
Standard calibration gasses were supplied by Airgas (+/- 0.2%) and were fed both directly to the GA4 and through a BioBLU 1f Single-Use Vessel containing 1L of sterile phosphate buffer. The gas was supplied at a steady flow rate of 40sL/h for 7 hours to monitor the stability of the reading over time.

Biological tests for two ATCC® organisms, *Bacillus subtilis* (ATCC 6051) and *Pseudomonas vancouverensis* (ATCC 700688) were conducted in the BioBLU Single-Use Vessel using the ATCC recommended media and temperature, 1 VVM air flow, agitation based DO control (30% min), and acid/base pH control (7.0, phosphoric acid, NaOH).

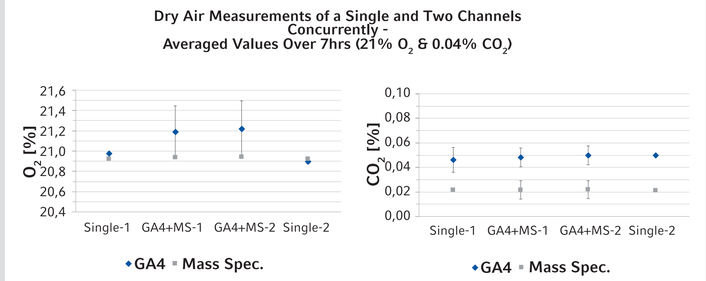


Equipment orientation and gas flow diagram

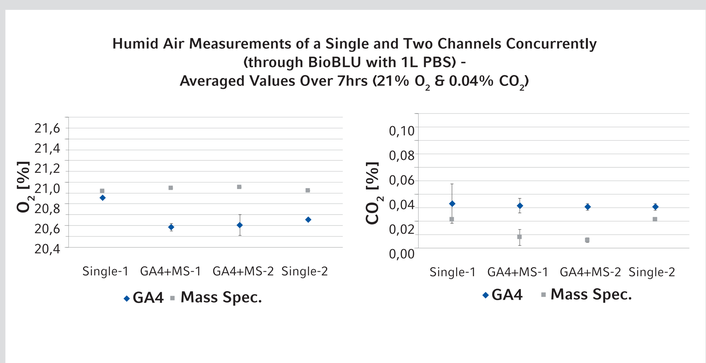
Results



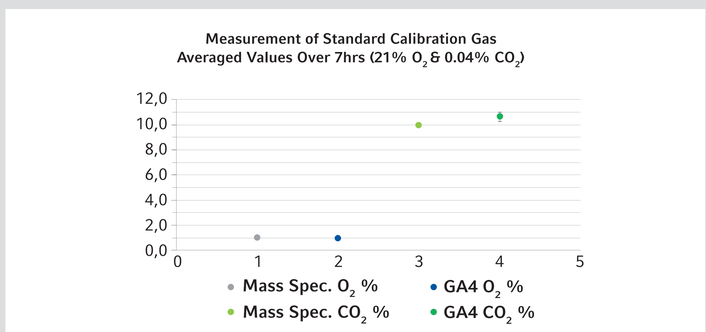
Gas flow measurements from both units demonstrate little interference when two instruments are in the same flow



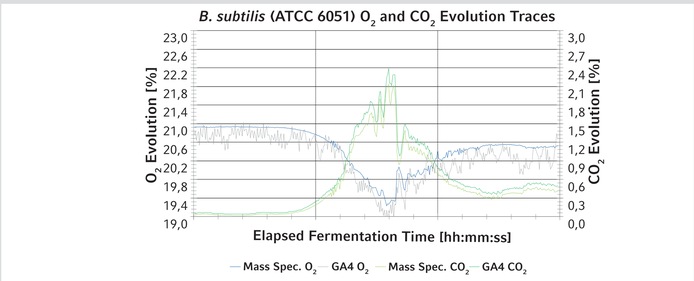
The addition of the mass spectrometer to the gas stream did introduce some variation in the off gas measurements of the GA4. This is attributed to the increased back pressure caused by the mass spectrometer's sampling apparatus. However measurements were still less than ± 0.5%. When measuring with multiple channels, the results were similar, indicating no interference when multiple units were measured at once

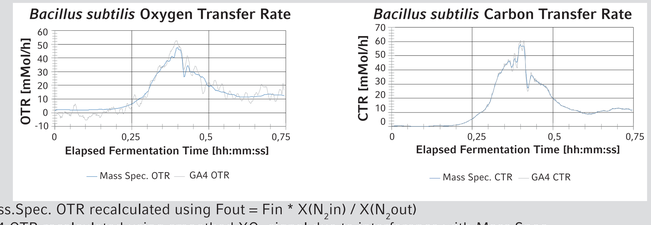


When the gas supply was routed through a bioreactor containing 1L of PBS, the GA4 measures lower amount of oxygen, caused by the increased water vapor which will be compensated for the later OTR and CTR calculations. Variation between GA4's values and the mass spectrometer were still present (< ± 0.5%)

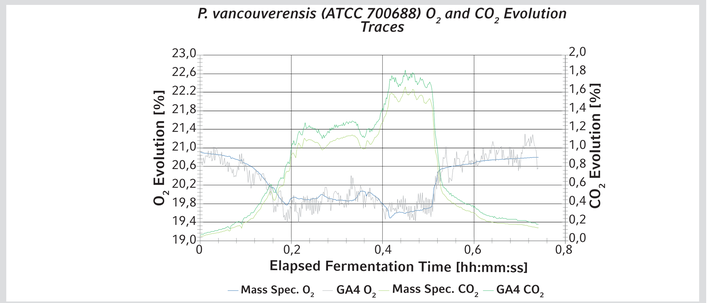


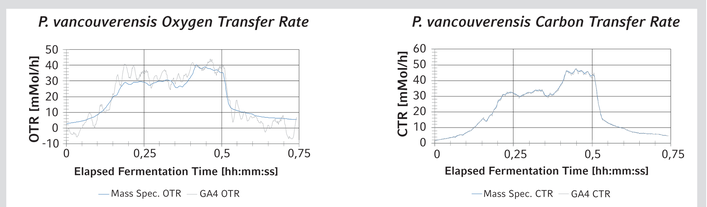
Measurements of three different standard gasses all showed high correlation between the GA4 and the mass spectrometer (0% O_2 with 20% CO_2, and 15% O_2 with 5% CO_2 results not shown)



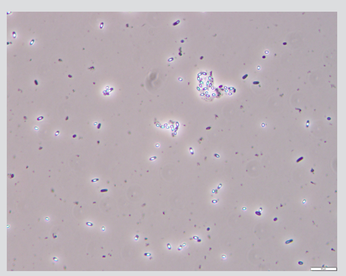


Mass.Spec. OTR recalculated using Fout = Fin * X(N_2in) / X(N_2out)
GA4 OTR recalculated using smoothed XO_2 signal due to interference with Mass.Spec.

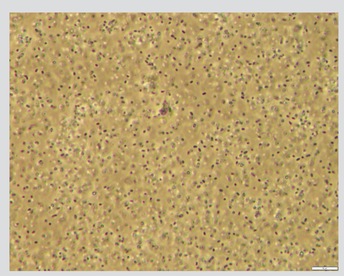




Fermentations of two model strains showed consistent O_2, CO_2 and OTR, CTR values (< ± 0.5%), while also closely matching one another during all phases of growth including lag, exponential, and stationary. In addition, smaller changes in metabolism indicated by changing O_2 and CO_2 evolution were captured by both units.



B. subtilis (ATCC 6051) 100x phase contrast microscopy – 48hrs EFT



P. vancouverensis (ATCC 700688) 100x phase contrast microscopy – 48hrs EFT

Conclusion

> Off gas values and trends from the DASGIP GA4 and Thermo-Fisher Prima Pro were comparable for Air, standard gasses, and biological experiments.
> Having the GA4 and the Mass Spec. in the measuring stream leads to interference with the O_2 and CO_2 measurements of the GA4 due to the MS' systems backpressure.
> The GA4 and mass spectrometer can be used in conjunction with each other, if additional volatile compounds, such as ethanol wished to be measured using the mass spectrometer.
> The GA4's integration into the DASGIP system allows for automatic, real time calculation of oxygen transfer rate, carbon evolution rate, and the respiratory quotient, due to its compensation strategies with regards bioreactor volume, exhaust gas humidity and exhaust gas flow. These calculations must be done manually for the mass spectrometer.
> Manual calculations can be done DASware control's scripting opportunity.

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