ROBUST, ACCURATE OFF-GAS ANALYZERS DESIGNED FOR USE IN CULTURE BIOSCIENCES HIGH-THROUGHPUT BIOREACTOR FACILITY

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ABSTRACT

On-line analysis of off-gas composition (CO_2 and O_2) is crucial for understanding and optimizing bioprocesses. Culture Biosciences has integrated off-gas analyzers into every bioreactor in their high-throughput fermentation system, allowing continuous tracking of important metabolic information such as OUR and CER. Here, the performance and stability of Culture Bioscience's custom-made analyzers is assessed and compared with a comparable commercially available off-gas analyzer.

INTRODUCTION

The online measurement of off-gas in bioreactors is a well-established practice in industrial biomanufacturing[1]. Analysis of gas composition (O_2 and CO_2) enables the calculation of key bioprocess variables: oxygen uptake rate (OUR), carbon dioxide evolution rate (CER), and respiratory quotient (RQ). These in turn provide essential information that can be used for process optimization, control, scaling, and validation.

Process optimization: At the most fundamental level, off-gas analysis enables more accurate computation of mass balance and carbon balance. A detailed assessment of OUR and CER, calculated from raw off-gas measurements, can lead to a better understanding of cellular metabolism and specific growth rate, which in

turn can inform optimization of biomass generation, product titer, and product quality[2].

Process control: Off-gas sensor data can be used in feedback control loops for maintaining specific growth rates in the bioreactor. Continuous online measurement of off-gas concentration allows for greater precision through the use of automated control of process parameters in real-time.

Process scaling: Oxygen consumption rates, calculated from raw off-gas data, are used in determining the oxygen transfer rates, which are an important scale-up parameter.

Process validation: Off-gas analysis is widely available on industrial scale equipment and can be an important part of commercial process validation and control. Specifically, certain PAT (process analytical technology) control strategies depend on an understanding of off-gas metrics and their relationship with biochemical processes occurring within the bioreactor[1].

Given the critical importance of real-time off-gas data for bioprocess optimization and scale-up, Culture Biosciences designed off-gas analysis units to track O2 concentration, CO2 concentration, temperature, and humidity.

Each bioreactor contains an independent off-gas analysis unit, allowing for fast sampling times with no delays and no multiplexing.

MATERIAL AND METHODS

Each off-gas analysis unit on Culture Bioscience's bioreactors consists of three separate digital sensors: one measuring O_2 concentration, one measuring CO_2 concentration, and one measuring both humidity and temperature. These three sensors are all commercially available OEM components. The details of each sensor can be found in the Table of specifications below (**Table 1**).

The sensors are housed inside flow-cell units and are connected in series with bioreactor exhaust going through first the humidity sensor, then the O_2 sensor, then the CO_2 sensor. This connection of the sensors together in a direct flow path from the bioreactor minimizes response time, which is important for accurate detection at low gas flow rates. A condenser at the exhaust port of the bioreactor protects the sensors from high levels of water vapor, and a 0.2um sterile filter in the exhaust line protects the off-gas sensors from particulates.

	Humidity and Temperature Sensor	O_2 Sensor	CO_2 Sensor
Technology	Capacitive and resistive	Optical (luminescence quenching)	Non-dispersive IR
Range	0-100% RH non-condensing 20-50˚C	0-25%	0-20%
Accuracy	1.5% RH 0.1˚C	<2% of full scale (0.5% absolute)	+/- 70ppm or 5% relative
Response Time	<10 s		
Operating Temp.	-40-105˚C	-30-60˚C	0-50˚C
Operating Pressure	N/A	0.5-1.2 bar	0.5-2.0 bar
Operating Humidity	0-100% RH non-condensing	0-99% non-condensing	0-95% non-condensing

Table 1. Sensor Specifications

RESULTS AND DISCUSSION

Calibration and Stability

The O_2 and CO_2 sensors used in the off-gas analysis units are factory calibrated from the manufacturer. However, before use in Culture Bioscience's reactors, a zero point calibration with nitrogen gas, and a custom gas mixture calibration with 10.00% CO_2 in air are performed.

The stability of the sensors was investigated by running them in a continuous series of fermentations with both *E. coli* and *S. cerevisiae* over 40+ days. Two O_2 sensors and two CO_2 sensors were used for this investigation. At certain intervals over the 40+ days of continuous fermentation, a calibration gas was used to determine the relative drift from their original reading. The data from this assessment of calibration factor relative offset is shown in **Figure 1.**

Throughout the entire 40+ day test period, the O_2 sensors showed less than 2% relative drift, while the CO_2 sensors showed less than 4% relative drift. To maintain high accuracy of off-gas measurements in Culture Bioscience's high-throughput fermentation system, the off-gas analysis units are calibrated every 30 days.

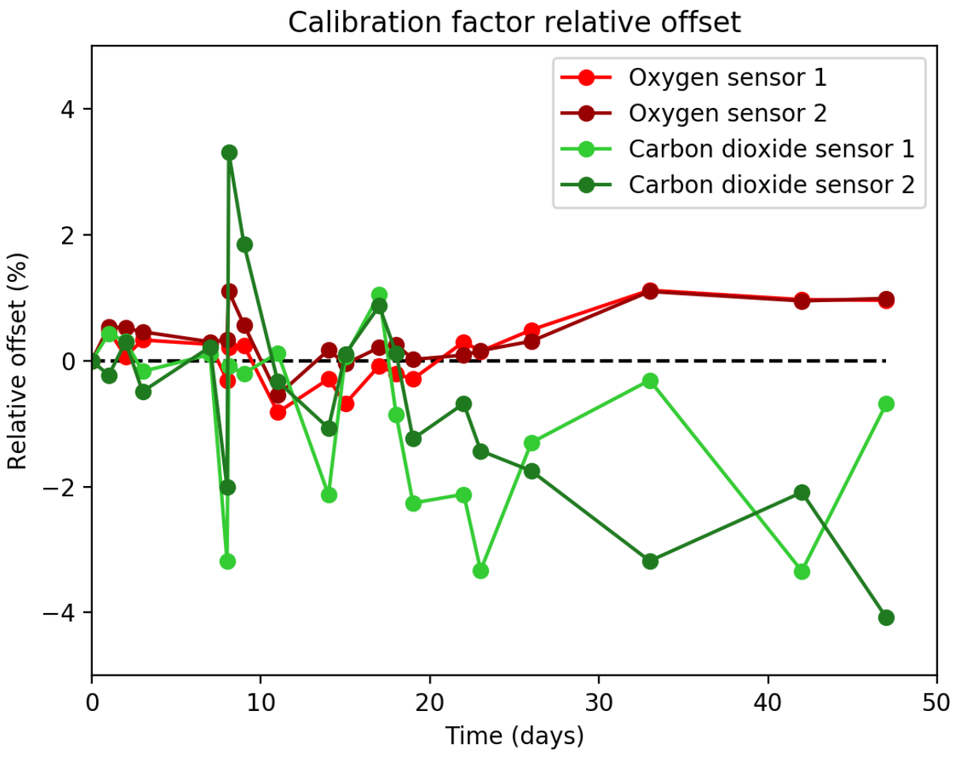
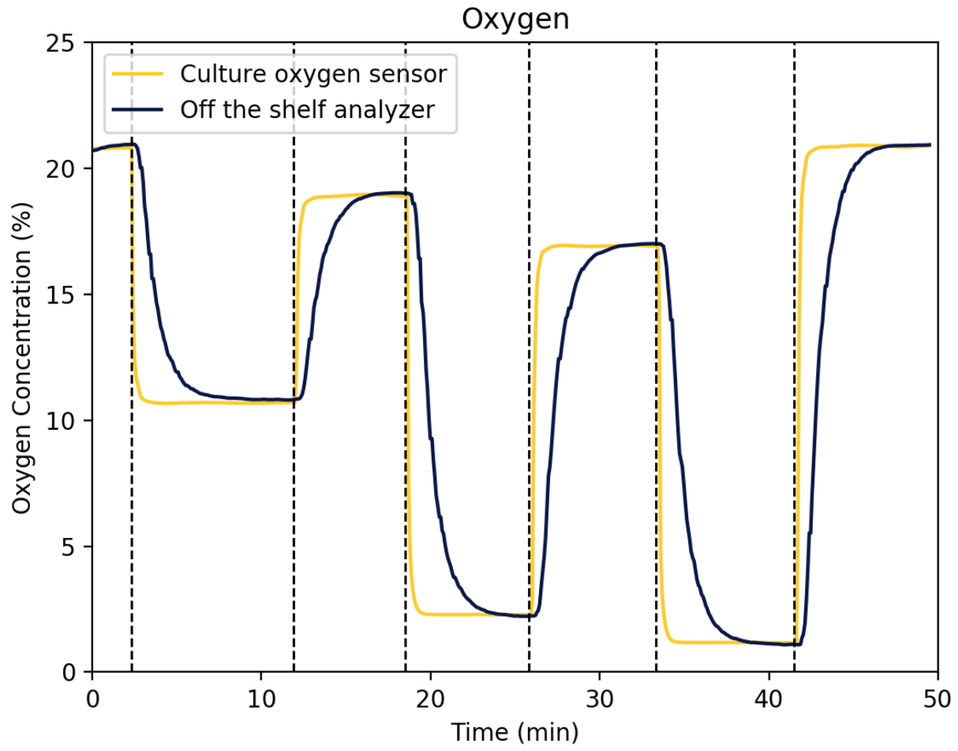


Figure 1. Relative drift of two O_2 and two CO_2 sensors over 40+ days while running continuously in active microbial fermentations.

Comparison to Off the Shelf Gas Analyzer

To ensure the accuracy and robustness of data generated by Culture Bioscience's off-gas analysis units, they were compared to a commercially available analyzer designed for use in fermentation systems. Dry gas mixtures were run through the off the shelf analyzer and Culture Bioscience's off-gas unit in parallel at 1 L/min to compare their accuracy and response time. The results of this comparison, where both units were properly calibrated before testing, are shown in **Figure 2**, below.



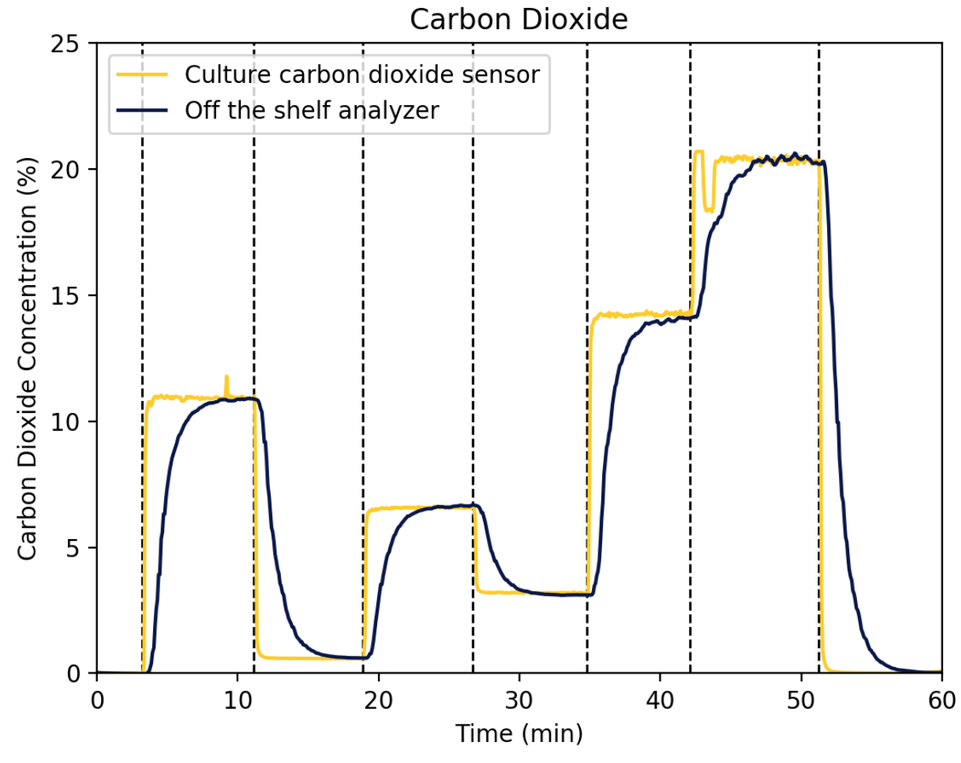


Figure 2. O_2 and CO_2 concentration of dry gas mixture step changes recorded by Culture Bioscience's off-gas analyzer (yellow) and a commercially available gas analyzer (blue). Step changes in gas concentration are marked with dashed vertical lines.

Both the off the shelf analyzer and Culture Bioscience's off-gas units show the same steady state concentration values within 2% relative error. The response time of Culture Bioscience's off-gas analyzer, which uses a direct flow-through system, was slightly faster than the off the shelf analyzer.

Culture Bioscience's off-gas analyzers were also tested head-to-head against the off the shelf unit in a live microbial fermentation with *S. cerevisiae*. The off the shelf analyzer was put first in series from the exhaust line from the bioreactor, with Culture Bioscience's analyzer second in series. The raw gas concentrations and the relative offset between the two different analyzers are shown in **Figure 3**.

The results of this comparison show that Culture Bioscience's off-gas analyzer behaves almost identically to the off the shelf analyzer when used in a typical fermentation process (i.e. typical aeration rate and headspace containing water vapor, volatile metabolites, etc.).

Effect of Organic Vapors

Many microbial fermentation systems contain organic compounds such as ethanol and methanol that may interfere with the accuracy of off-gas sensor measurements. Therefore, it was critical to ensure that the effect of organic vapors' interference with Culture Bioscience's off-gas analyzers was minimal. To test the sensitivity of the sensors to organic vapors from the bioreactor,

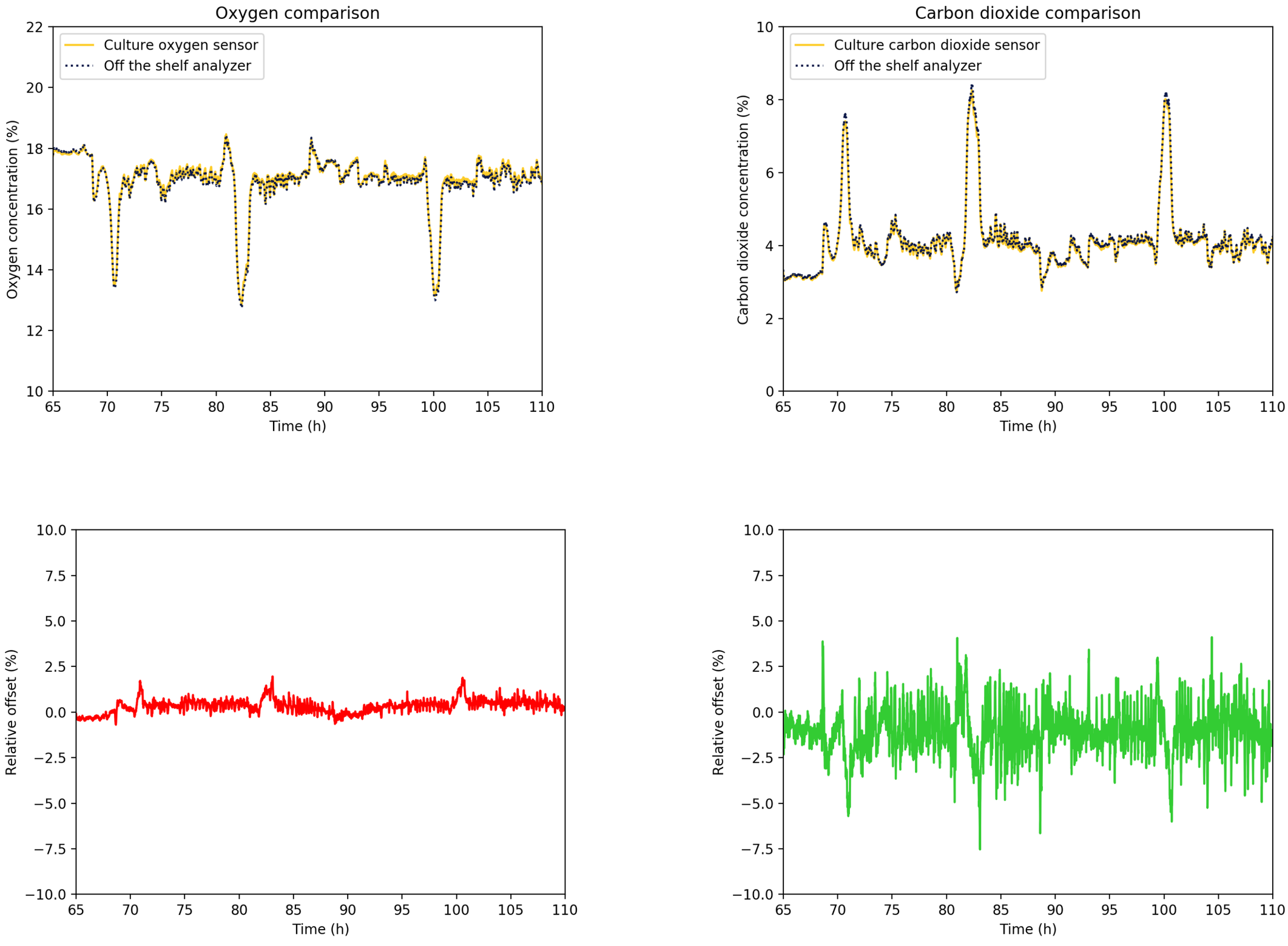


Figure 3. O_2 and CO_2 concentration of live yeast fermentations recorded by Culture Bioscience's off-gas analyzer and a commercially available off-gas analyzer. The relative percent offset from the Culture sensor and the off the shelf unit are plotted in the second row.

CO_2 in air mixtures were bubbled through 0, 0.5, and 5.0 wt% solutions of ethanol in water at 0.1 L/min at 37°C and 1000 rpm agitation. Additional tests with different mixtures of CO_2 in air (5 and 10 vol%) and O_2 in nitrogen (20.5% and 18.85%) were also conducted.

As seen from the results in **Table 2**, there was less than 4% relative drift in CO_2 and less than 2% relative drift in O_2 values between 0 and 5 wt% ethanol in water. Typical fermentations would have much lower than 5 wt% of volatile organics. A similar study where air was bubbled through water solutions with up to 10% wt methanol showed less than 1% relative drift in O_2 values and less than 0.02% absolute drift in CO_2 values (data not shown).

OUR, CER, and RQ Calculators

OUR (oxygen uptake rate), CER (carbon dioxide evolution rate), and RQ (respiratory quotient) can all be calculated by using the raw off-gas concentration data generated by the sensors in combination with gas flow rate data and volume estimations. These calculations are reported live on Culture Bioscience's online data monitoring platform during fermentations. **Figure 4** shows the OUR, CER, and RQ data for an *E. coli* fermentation with a DO (dissolved oxygen)
spike feeding strategy.

The cells were grown in batch phase until a DO spike at 7-7.5 hours EFT, which can be seen in the sharp spike in DO and sharp drop in OUR. Cells were then fed with 500 g/L glucose when a DO spike of above 30% was detected.

The oscillations in metabolism from this spike feeding strategy can be seen in the OUR data. The RQ is between 0.95-1.05, as expected for *E. coli* fed on glucose.

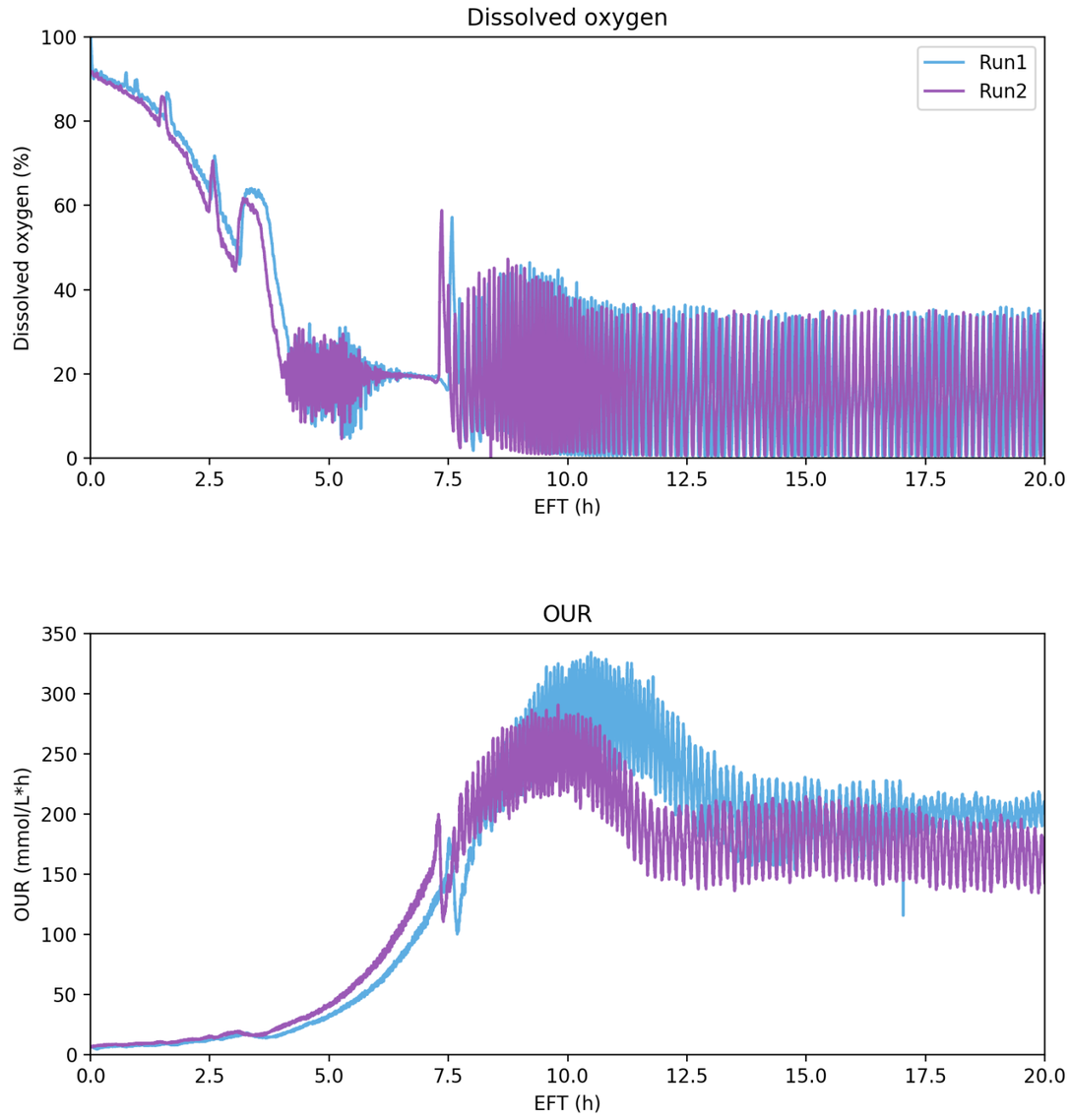


Figure 4. OUR, CER, and RQ of E. coli runs with DO spike feed control.

Table 2. Effect of ethanol vapors on O_2 and CO_2 concentration in various gas mixtures

Mass % EtOH in water	Relative % drift compared to 0 wt% EtOH reading			
	5% CO_2	10% CO_2	20.5% O_2	18.85% O_2
0.5%	0.79%	0.08%	0.00%	0.21%
5%	3.00%	3.37%	1.24%	1.49%

CONCLUSION

Strain engineers and upstream bioprocess engineers are frequently required to work under tight timelines to optimize bioprocesses. Off-gas data and the key parameters calculated from it enable a deeper understanding of bioprocesses that can enable researchers to develop and scale-up their processes faster. The ability to conduct high-throughput strain screens or process development DOEs at Culture Biosciences can further accelerate R&D timelines. Scientists can now generate more data than ever about their bioprocess with robust, accurate off-gas analysis for each fermentation run in Culture Bioscience's benchtop bioreactors. See **Figure 5** for an example of a high-throughput process development DOE with 52 bioreactors run in parallel.

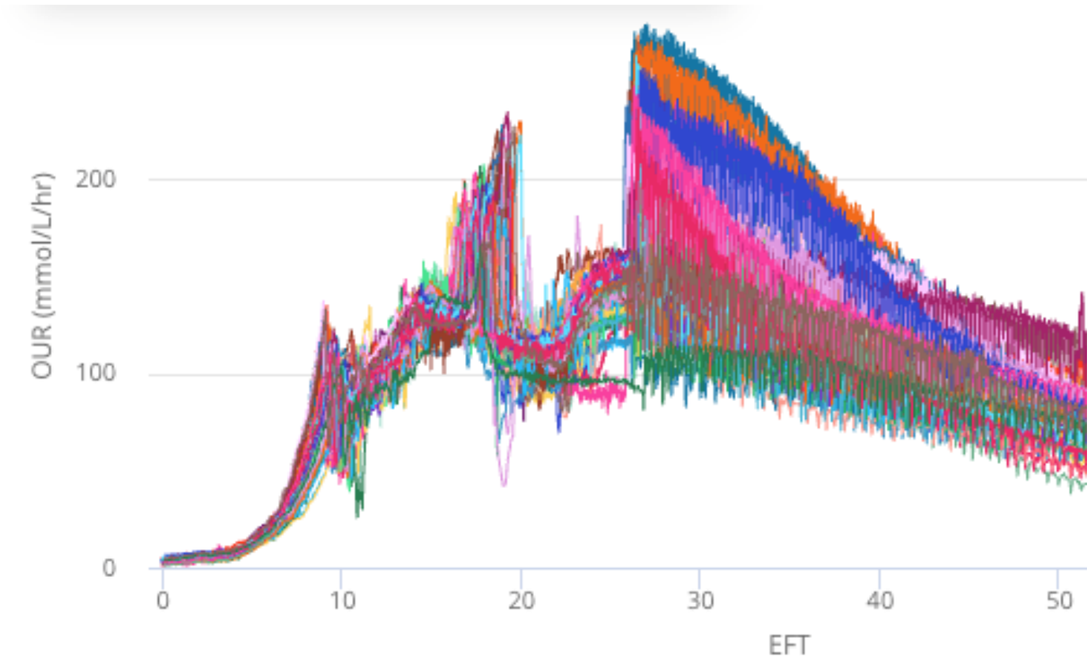


Figure 5. Oxygen uptake rate (OUR) from the 52 parallel bioreactor runs. This graph was auto-generated using live streaming data from Culture Bioscience's cloud-based online monitoring technology.

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