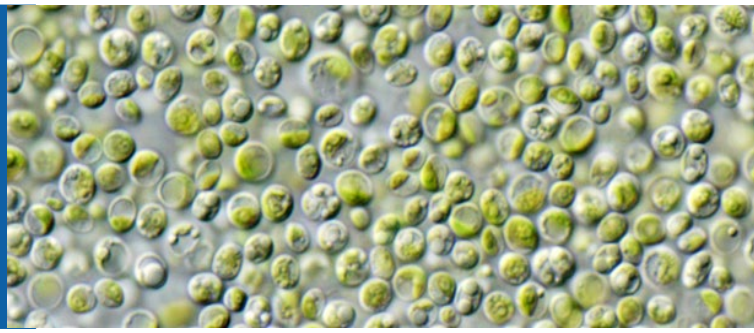
Continuous Online Microalgae Biomass Monitoring



Microalgae cultivation is a growing area of biotechnological interest, with applications spanning nutraceuticals, animal feed, biofuels, and high-value specialty compounds. Like other cell culture processes, precise monitoring of biomass concentration is essential to understanding growth dynamics, optimizing feed strategies, and determining harvest endpoints. Without continuous inline measurement, operators must rely on periodic manual sampling—a labor-intensive approach that leaves gaps in the process record, introduces contamination risk, and provides no real-time visibility between sampling events.

This application note demonstrates how the Exner EXcell NIR absorption probe addresses these challenges, providing continuous, quantitative biomass measurement throughout extended photobioreactor cultivation runs. Chlorella sorokiniana, a fast-growing green microalga with high temperature tolerance, was selected as the model organism, cultivated in a Subitec 6-liter flat-panel airlift photobioreactor (FPA-PBR) operated in repeated fed-batch mode.

THE MEASUREMENT CHALLENGE

Photobioreactors present a specific set of challenges for inline optical measurement. The culture medium is continuously sparged with gas to supply CO_2 and maintain mixing, generating bubbles that can interfere with optical signals. Biomass concentration changes continuously and often rapidly, and the characteristic repeated fed-batch operating mode with regular dilution steps requires a sensor capable of tracking both growth and step changes in real time. Low biomass concentrations in the early growth phase must be resolved accurately, while the sensor must also remain reliable as density increases toward harvest.

The Exner EXcell probe was selected for this application based on its NIR operating wavelength, compact in-situ design, and proven performance across a wide biomass measurement range.

EXPERIMENTAL SETUP

The Subitec FPA-PBR is a fully automated cultivation platform in which temperature, light intensity, and aeration rate can be independently controlled. The Exner EXcell NIR absorption probe was installed directly in the reactor, configured with a 10 mm path length optical gap. Operating in the near-infrared wavelength range, the EXcell minimizes interference from the natural pigmentation of the algal medium, an important consideration in microalgae applications where medium color would compromise measurements made at visible wavelengths.

Subitec system and Exner EXcell Probe

The digital sensor signal was transmitted continuously via the Exner ECI 03 communications interface to the facility's internal process control system, where data was logged at regular intervals throughout each cultivation run.

To establish a quantitative biomass calibration, daily offline samples were analyzed using a benchtop photometer to determine optical density, which was then converted to dry cell weight (DCW, g/L) using an empirically established OD-to-DCW correlation specific to Chlorella sorokiniana. These offline reference values were used to correlate against the inline EXcell absorbance signal, expressed in Absorbance Units (AU), confirming the probe's ability to serve as a continuous, calibrated biomass monitor.

FROM LAB-SCALE DEVELOPMENT TO PRODUCTION

Trial 1

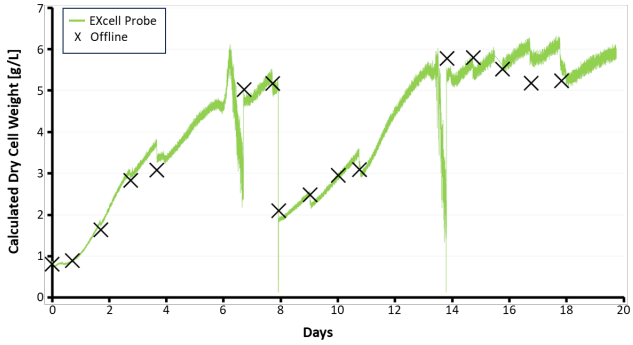


Figure 1: *Cultivation trend for Trial 1. The continuous Exner EXcell inline sensor signal (green trace, AU) is shown alongside daily offline dry cell weight reference measurements (×). The close agreement between inline and offline values throughout the 21-day run confirms the probe's accuracy across the full growth curve, from inoculation through peak biomass. Sharp downward spikes reflect deliberate dilution events and weekend evaporation periods where the probe was temporarily exposed to the reactor headspace.*

Over a 21-day cultivation period, the Exner EXcell probe continuously tracked the biomass profile of the culture with high fidelity. The characteristic sawtooth pattern visible in the trend data directly reflects the repeated fed-batch operating strategy: each daily addition of fresh medium to compensate for evaporative losses produces a step reduction in biomass concentration, captured in real time by the probe, followed by a clear resumption of exponential growth. This level of process resolution would not be achievable through periodic manual sampling alone.

Transient signal excursions observed between days 6–7 and days 13–14 were caused by the probe tip being exposed to the reactor headspace during periods of elevated evaporation over the weekend. This is a setup-specific condition unrelated to probe performance. On day 8, a deliberate dilution of the culture to approximately 1.9 g/L was clearly captured as a sharp step change in the EXcell signal, immediately followed by resumed growth tracking.

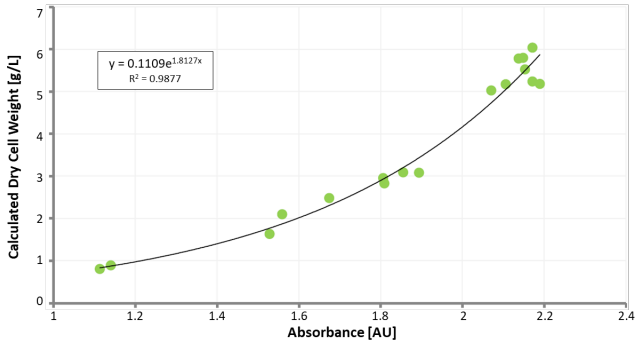


Figure 2*: Correlation of Exner EXcell inline sensor signal (AU, x-axis) versus offline dry cell weight (g/L, y-axis) for Trial 1. The data follows the exponential relationship y = 0.1109e^1.8127x with R² = 0.9877, confirming a strong, reliable correlation between the inline probe signal and gravimetric biomass concentration across the full measurement range.*

Peak biomass concentration reached approximately 6.2 g/L DCW, corresponding to an EXcell absorbance reading of 2.2 AU. Correlation of daily offline OD/DCW values against the continuous inline EXcell signal demonstrated a strong exponential relationship with a coefficient of determination of $R^2 = 0.9877$.

Trial 2

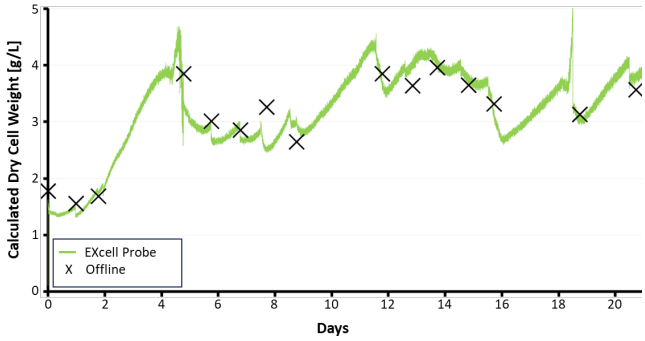


Figure 3*: Cultivation trend for Trial 2. The continuous Exner EXcell inline sensor signal (green trace, AU) is shown alongside daily offline dry cell weight reference measurements (×). Trial 2 exhibits a more complex biomass profile than Trial 1, demonstrating the EXcell probe's ability to continuously and accurately track dynamic process conditions — growth, decline, and recovery phases alike — that would be largely invisible from daily offline sampling alone. The close agreement between inline and offline reference points throughout the run confirms reliable probe performance, with correlation yielding $R^2 > 0.99$. Sharp downward spikes reflect brief periods of probe headspace exposure during evaporative losses.*

The second run confirmed the repeatability of the EXcell probe's performance across an independent cultivation campaign. The probe again accurately tracked all growth and dilution events throughout the 21-day period. Peak biomass in this run reached 4.3 g/L DCW on day 11. This was somewhat lower than Trial 1 and reflects differences in cultivation conditions. Despite this, the EXcell signal faithfully reflecting the reduced growth trajectory. Signal artifacts on days 5 and 18 were again attributable to probe headspace exposure during evaporative periods.

Correlation of offline and inline data in Trial 2 produced an even stronger agreement than Trial 1, with $R^2 > 0.99$, further validating the EXcell probe as a reliable quantitative biomass sensor for this application.

DISCUSSION

The results of both cultivation runs highlight several important performance characteristics of the Exner EXcell probe that make it particularly well suited to microalgae photobioreactor applications.

Sensitivity at low biomass concentrations. The EXcell probe reliably resolved biomass concentrations below 1.0 g/L DCW, a range critical for monitoring early-phase growth and detecting lag phase behavior. Many optical sensors struggle at low cell densities; the EXcell's NIR design and 10 mm path length configuration provided sufficient sensitivity to track growth from inoculation onward.

Wide usable measurement range. Peak biomass concentrations of 6.2 g/L in these trials corresponded to only 2.2 AU out of a maximum probe range of 6 AU, indicating that the EXcell was operating well within its linear range throughout both studies. Based on this headroom, biomass concentrations in excess of 20 g/L DCW are expected to remain within the probe's reliable measurement range, making it suitable for higher-productivity process development without sensor substitution.

Real-time resolution of process events. The EXcell's continuous inline signal captured deliberate dilution steps, evaporative concentration effects, and growth rate variations with a resolution and immediacy that periodic offline sampling cannot match. This is particularly valuable in repeated fed-batch processes, where understanding the response to each feeding event is central to process optimization.

Measurement deviations. Minor deviations of up to 0.5 g/L observed at certain time points (Trial 1, days 8 and 13; Trial 2, day 17) were attributed to variability inherent in the manual offline photometer measurements used as reference values, rather than to any limitation of the inline probe. With the EXcell's onboard smoothing function set to level 9 (of 100), clean, well-resolved trend data was obtained without masking genuine process dynamics.

NIR wavelength advantage. The use of NIR light eliminates the significant optical interference caused by the green pigmentation of Chlorella cultures, which would produce unreliable readings at the visible wavelengths used by many standard photometers. This makes the EXcell inherently better suited to pigmented microalgae applications than broadband or visible-wavelength alternatives.

CONCLUSIONS

This study demonstrates that the Exner EXcell NIR absorption probe is a highly capable solution for continuous inline biomass monitoring in microalgae photobioreactor applications. Across two independent repeated fed-batch cultivation runs totaling over 40 days of operation, the probe delivered accurate, continuous biomass data with strong correlation to offline reference measurements ($R^2 > 0.985$ and $R^2 > 0.99$ respectively).

By providing an unbroken biomass record throughout each cultivation, including overnight and weekend periods when manual sampling is impractical, the EXcell probe dramatically reduces reliance on routine offline sampling while delivering superior process insight. Its compact, in-situ design, NIR wavelength selection, wide dynamic range, and simple digital communications make it a practical and scalable solution from laboratory-scale development through to production.

This document is a summary of a collaborative application study carried out by Subitec GmbH & Exner GmbH

- Exner EXcell probes are available in 12mm and DN25 formats, and fit most benchtop, pilot, and production scale bioreactors..
- Optical Pathlengths can be selected for the best resolution of measurement depending upon cell density

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