# From $CO_2$ measurement to isotope-resolved analysis

Respirometry is a key analytical approach for evaluating biodegradation of plastics and other organic materials across a wide range of environmental conditions, including compost, soil, and aqueous systems. In standardized aerobic biodegradation testing, mineralization is typically quantified by measuring the amount of evolved carbon dioxide, as described in methods such as ISO 14855-1 for controlled composting conditions.

Recent advances in instrumentation (especially new sensor development) have extended respirometry from endpoint-based testing toward continuous kinetic analysis and flexible application across research and industrial settings. Modular multi-channel and flow-through respirometry systems enable both rapid screening and detailed biodegradation studies under controlled conditions.

Modern respirometry systems have evolved significantly over the past decade. Traditional closed systems based on pressure measurement are increasingly complemented by open or flow-through systems that directly measure gas composition. This shift has enabled more accurate and application-specific testing, particularly for solid materials such as plastics.

This article reviews the application of Echo Instruments XC and ER series across diverse biodegradation scenarios. XC Respirometer is suited for screening and research respirometry applications at lower temperatures, while the ER Respirometer provides continuous monitoring of $CO_2$ and $O_2$ in higher temperature ranges. Both systems enable detailed kinetic analysis and automated biodegradation calculations.

The integration of respirometry with stable isotope analysis is a new technological approach. By coupling respirometry systems with cavity ring-down spectroscopy (CRDS) isotope analysers, it is possible to monitor the isotopic composition ($\delta^{13}C$) of evolved $CO_2$ and distinguish between carbon originating from the test material and background organic matter. This approach improves interpretability in complex systems such as compost and soil. Isotope-resolved respirometry does not accelerate biodegradation itself but enables earlier and more specific detection of material-derived mineralization.

## Introduction

The growing use of biodegradable polymers has increased the need for reliable, application-specific biodegradation testing methods. Materials such as polylactic acid (PLA), polybutylene succinate (PBS), and starch-based blends are widely marketed as biodegradable; however, their actual degradation behaviour depends strongly on environmental conditions and material properties.

Biodegradation testing is therefore performed under a range of standardized conditions designed to simulate different environments, including compost, soil, and aquatic systems. Among the available analytical approaches, respirometry has become one of the most widely used techniques due to its ability to directly quantify microbial activity.

Respirometry methods are based on measuring oxygen consumption or carbon dioxide evolution during microbial degradation. For plastics, $CO_2$-based methods are particularly relevant, as they directly reflect mineralization of carbon in the test material. Standards such as ISO 14855-1 define biodegradation as the conversion of organic carbon into $CO_2$ under controlled composting conditions.

## Principles of respirometric biodegradation measurement

Under aerobic conditions, microbial degradation of organic material results in the formation of carbon dioxide, water, and biomass. Respirometry quantifies this process by measuring either oxygen uptake or $CO_2$ evolution.

In composting and solid-material applications, $CO_2$ evolution is generally preferred because it directly reflects carbon mineralization. The resulting biodegradation curve typically follows a characteristic pattern:

- Lag phase – limited activity during initial adaptation and hydrolysis
- Active phase – rapid microbial degradation and $CO_2$ production
- Plateau phase – stabilization as the substrate is depleted

This sigmoidal behaviour is well-established in standardized biodegradation testing and provides insight into both the rate and extent of biodegradation.

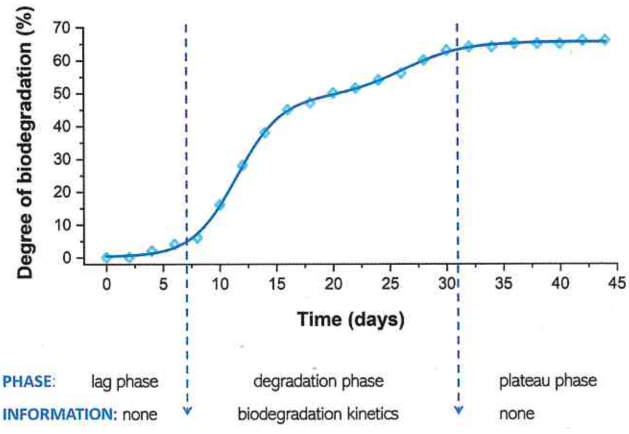


*Figure 1:*
*Respirogram showing bioplastic degradation over time with phase information.*

## Echo respirometry systems

### XC Series – screening and method development

The XC Respirometer is an extra-compact miniature modular platform designed for research and development applications. It can be integrated with different reactor configurations and laboratory setups, making it suitable

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for rapid screening of materials, comparative testing, and optimization of experimental conditions. Its flexibility allows researchers to explore multiple variables efficiently, which is particularly valuable in early-stage material development.


*Figure 2:*
*XC Respirometer with various measurement vessels*

**ER Series – controlled and continuous measurement**

The ER Respirometer is a flow-through respirometry system designed for continuous and controlled measurement of biodegradation at high temperatures, simulating composting conditions in detail. It monitors: $CO_2$ concentration, $O_2$ concentration, gas flow with MFC, temperature, pressure, and humidity. The system enables automated calculation of cumulative $CO_2$ production and the percentage of biodegradation. Due to its stable gas flow and controlled conditions, it is well suited for biodegradation studies aligned with methods such as ISO 14855-1, ASTM D5338, and related protocols. This is a reliable work horse for long term analysis in any conditions. It can also be used in bioplastic production plants to monitor production processes, support quality control of input materials, track fermentation processes, and assess product biodegradability.


*Figure 3:*
*ER Respirometer control unit*

## Applications

### Composting

Controlled composting is one of the most important environments for evaluating biodegradable plastics. Under thermophilic conditions (~58 °C), microbial activity is high, enabling efficient mineralization of suitable materials. Respirometry is widely used to quantify biodegradation under these conditions by measuring $CO_2$ evolution. It allows comparison of different materials, forms, and processing conditions.

### Soil systems

Biodegradation in soil is typically slower and more variable than composting. Respirometry enables monitoring of microbial activity and material degradation under different conditions, although interpretation is more complex due to environmental variability. It is important to use well-defined, reproducible, or artificial soil.

Soil is also an important medium, particularly for mulch film analysis, where accurate determination of biodegradation is essential for field applications. Analytical studies of the biodegradation of individual components, as well as the final product, help optimize overall material performance.

### Aqueous systems

In aqueous systems, respirometry is used to assess long term biodegradation of bioplastic, soluble compounds, and additives. Oxygen uptake methods are commonly used, while $CO_2$-based approaches are increasingly applied for more detailed analysis. Echo Instruments XC Respirometers also enable continuous monitoring of plant or algal respiration by measuring $CO_2$ and $O_2$ dynamics over time. This allows quantification of daily metabolic cycles, growth-related changes, and physiological responses to light and nutrient availability. The method is also highly relevant for sediment studies in rivers, lakes, and marine environments, where flow conditions can be simulated without disturbing the sediment structure. The same system can also be used to test influence of different compounds on microorganisms, algae, or aquatic plants.

## Comparison of traditional and modern respirometric laboratory – new software from Echo Instruments

Traditional respirometric laboratory systems are typically designed for single-experiment operation, where one respirometer is dedicated to one set of reactors and one experiment under a single set of environmental conditions. This configuration limits flexibility, as expanding experimental capacity requires the acquisition of additional complete respirometry units. Furthermore, unused reactors remain idle until a new experimental setup is initiated, leading to inefficiencies in resource utilization. This system also restricts the use of references and standards to one per respirometer and confines operation to a single physical location.

In contrast, modern respirometric systems offer a scalable and integrated approach, allowing simultaneous control of multiple respirometers across one or more experiments, potentially at different locations. Reactors can be flexibly distributed among different systems, allowing unused reactors to be readily reassigned to new experiments without delay. This configuration supports parallel testing of multiple environmental conditions and allows multiple references and standards to be applied across interconnected systems. Additionally, modern platforms enable centralized and location-indĕpendent operation, effectively functioning as a unified, large-scale respirometry system. This approach significantly enhances experimental throughput, flexibility, and overall efficiency.


*Figure 4:*
*New software platform for optimal experiments organization*

## Integration with stable isotope analysis

Conventional respirometry provides quantitative information on total microbial activity through oxygen consumption or carbon dioxide evolution, but it does not inherently distinguish between different carbon sources contributing to the measured signal. This limitation becomes particularly relevant in complex systems such as compost or soil, where both the test material and the background organic matter are simultaneously biodegraded.

This challenge can be addressed by combining respirometry with stable isotope analysis.

By using $^{13}C$-labelled materials and measuring the isotopic composition ($\delta^{13}C$) of evolved $CO_2$, it becomes possible to separate contributions from the test material and background organic matter. In a combined setup, the gas outlet from the respirometer is connected to an isotope analyser. The respirometer provides controlled airflow and total $CO_2$ measurement, while the analyser continuously measures $\delta^{13}C$ of the $CO_2$ stream.

A Picarro analyser measures the isotopic composition of $CO_2$ in real time, typically expressed as $\delta^{13}C$ values relative to a standard. By continuously monitoring $\delta^{13}C$ alongside total $CO_2$ concentration, it becomes possible to partition the measured $CO_2$ into contributions from the test material (e.g. PLA) and the background organic matter (compost or soil). This configuration enables simultaneous monitoring of total mineralization and carbon source identity. The main advantage of isotope-resolved respirometry is improved interpretability. Unlike classical methods, which rely on blank correction, isotope analysis provides direct source partitioning. Echo Instruments expects that this will be a promising future technique for accurate and fast screening tests of biodegradation.

The main advantage is that isotope-resolved methods can detect biodegradation earlier, because changes in isotopic composition can be observed even when total $CO_2$ increase is small.

Classical $CO_2$-based respirometry remains the foundation of standardized biodegradation testing. It provides robust and reproducible measurements of mineralization and is fully suitable for regulatory and industrial applications. However, in complex systems such as compost and soil, background respiration can obscure early-stage biodegradation. In such cases, isotope-resolved respirometry offers significant advantages.

The combination of respirometry systems with isotope analysis therefore represents a powerful approach for future advanced biodegradation studies.

| Result | What it means | Instruments |
|---|---|---|
| Disintegration (ISO 16929) | Material breaks into small pieces | DT Respirometer |
| Respirometry (ISO 14855) | Carbon converted to $CO_2$ calculated on-line biodegradation | ER Respirometer |
| $^{13}C$ analysis | Confirms carbon origin measured in lag phase | Respirometer + $^{13}C$ analyser |

*Table 1:*
*Methods and instrumentation for studying PLA biodegradation*

## Conclusion

Respirometry remains a central tool for biodegradation assessment across multiple applications. Modern systems such as Echo XC and ER Respirometers enable both flexible screening and precise analytical measurement.

The integration of stable isotope analysis extends these capabilities by providing source-resolved insight into biodegradation processes. While classical respirometry remains essential for standard testing, isotope-resolved methods offer significant advantages for research and complex systems in the future. ■

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