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Measurement of biodegradation with automatic analyser systems and matrix problems

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Problem

Biodegradation measurement through respirometry relies on microorganisms in the compost, which act as natural sensors to detect and determine the degradation process. The variation in microbial types and populations within the compost can lead to measurement inconsistencies. It is particularly difficult to obtain compost with the same microbial activity and composition in different locations worldwide. As microorganisms in compost serve as sensors for biodegradation, the process is dependent on the compost's activity. In this study, activated vermiculite was used as an inoculum for PLA biodegradation to evaluate its effectiveness and potential as a compost alternative. By utilizing advanced analytical methods, it is possible to streamline biodegradation analysis and enhance its user-friendliness.

The plastic era and why we invented biodegradable plastics

Ages ago, people used natural materials like wood, leather, and linen that decomposed after use, maintaining a cleaner environment. However, with the invention of materials like Bakelite, PVC, polyethylene, epoxy resins, and other polymers, we entered the plastic era, causing substantial harm to the environment. The accumulation of plastic since the early 1900s is becoming increasingly evident, with growing impacts on ecosystems and human health. Our global goal should be to transition into a post-plastic era, where biodegradable plastics replace conventional ones, mimicking the decomposition of natural materials and leaving no environmental harm. This is the only way we can ensure a cleaner world, including unpolluted lakes, rivers, and oceans, for future generations.
To ensure the stated conditions, it is necessary to introduce special standards that regulate, which materials are allowed in certain environments and protect them from potential pollution. These standards should enable accurate biodegradation analysis of polymers in diverse ecosystems. It is essential that they simulate natural environments or allow for their automation through advanced analytical techniques. Additionally, these standards must be validated to assure consistent conditions throughout the entire biodegradation process. Most importantly, the key lies in how we interpret the data from instrumental measurements once decomposition in controlled environments is complete.

Standard ISO 14855 specifies a method for the determination of the ultimate aerobic biodegradability of plastics, based on organic compounds, under controlled composting conditions by measurement of the amount of carbon dioxide evolved and the degree of disintegration of the plastic at the end of the test. This method is designed to simulate typical aerobic composting conditions for the organic fraction of solid mixed municipal waste. The composting takes place in an environment wherein temperature, aeration and humidity are closely monitored and controlled. The test method is designed to yield the percentage conversion of the carbon in the test material to evolved carbon dioxide as well as the rate of conversion.

Sensors to perform biodegradation

Measuring the decomposition of bioplastics requires the use of sensors that can detect the process. A sensor is a device or module that can detect environmental changes and conveys them in a measurable way to a recording device. In the case of bioplastic biodegradation, the microorganisms in compost function as natural sensors. We could even say that respirometer is a large-scale microbial biosensor.
Compost, commonly used as an active medium for measuring biodegradability, hosts dominant microbial communities such as bacteria, yeasts, and actinomycetes that react with bioplastic (Figure 1). At low temperatures, up to 40 °C, mesophilic microorganisms predominate, and then easily degradable and soluble compounds, such as simple sugars, proteins, are quickly broken down. The main role at higher temperatures, between 43 °C and 67 °C, is assumed by thermophilic microorganisms, where they accelerate the decomposition of more complex compounds, such as fats, cellulose, lignin and other complex carbohydrates. Bacteria play a role in decomposition as mesophilic and thermophilic organisms, but each phase is dominated by a different type of bacteria. Fungi include molds and yeasts and are mainly responsible for the degradation of many complex polymers, allowing bacteria to continue the degradation process through the secretion of enzymes. Fungal species are numerous in both the mesophilic and thermophilic phases and are usually observed on the surface of the compost mixture. Although classified as filamentous bacteria, actinomycetes share similarities with fungi in terms of their growth habits and nutritional requirements. They represent an intermediate stage between bacteria and fungi and are commonly found in the later phases of composting, where they decompose complex organic compounds.
Composting is typically an aerobic process, meaning it requires a constant supply of oxygen. In addition, proper care must be taken to maintain the ideal pH and moisture levels, along with supplement the compost with necessary minerals to support the optimal growth of microorganisms. Accurate weighing of compost and sample, and good laboratory practice are also necessary to ensure high reproducibility and accuracy of measurements.

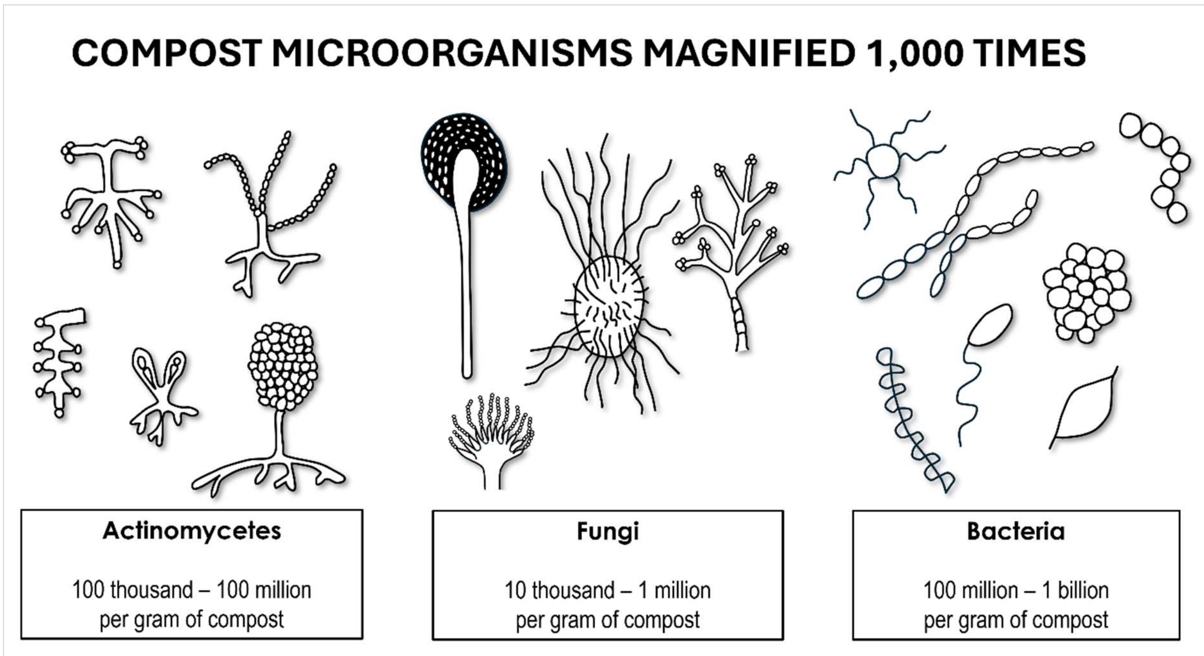

Figure 1*: Microorganisms in compost - sensors (Figure reproduced from Polymer Innovation Blog, Recycle and Disposal of Plastic Food Packaging Waste 3: How Compost Forms, by Jeffrey Gotro, Januar 16, 2017)*

Throughout our research, we found that compost compositions vary across the globe, making it difficult at times to provide equivalent conditions for biodegradation measurements in the respirometer.

Utilizing compost from industrial composting facilities that control accepted materials in accordance with established standards and certifications, such as ASTM D6400 and the Biodegradable Products Institute (BPI) certification, may prove beneficial in such situations.

Industrial composting is an actively managed process, where key factors are monitored to ensure effective and complete biodegradation. Compost managers monitor pH, carbon and nitrogen ratios, temperature, moisture levels, and more to maximize compost efficiency and quality, and to ensure adherence with regulations.

Key differences in these environments, which impact biodegradation, are varying microbial populations and activity, total organic carbon, temperature, moisture, as well as the levels of carbon, nitrogen, ammonia.

Given the challenges described, we sought a material that would offer greater reliability and allow for consistent measurements worldwide, irrespective of local conditions. For this reason, we selected vermiculite, a naturally occurring mineral, which is often used in gardening, construction, and various industrial applications.

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Automatic system for measuring biodegradation of bioplastic

Standards suggest utilizing uncomplicated laboratory equipment for biodegradation measurements to facilitate widespread use with limited resources. Automation of this process is advantageous and can be achieved with a measuring channel (Figure 2) in place to monitor and record all necessary physical and chemical parameters at both the entry and exit of the reactor vessel, making the process more user-friendly.

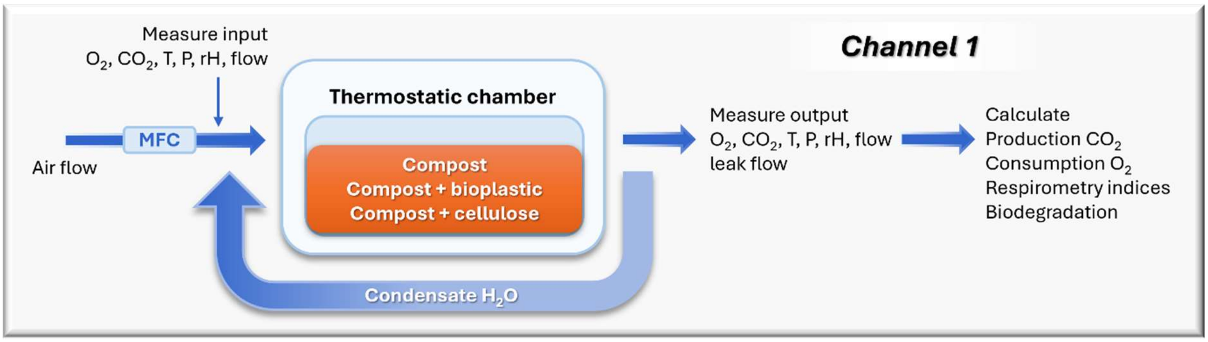

Figure 2: *Measuring channel for measuring biodegradation with an automatic system.*

By increasing the number of measurement channels to e.g. 60, we can analyse a batch of samples simultaneously using one device (Figure 3). Advanced computer software then controls all measurement parameters, providing real-time representation and tracking of biodegradation progress. Connecting multiple respirometers together is possible, allowing them to operate as a single measuring system. This flexibility allows us to configure the respirometers in the laboratory according to our specific requirements.
The PCI-controlled mass flow controllers maintain aerobic conditions in each reactor, and the automatic system keeps sample dehumidification minimal by returning the condensate.

Figure 3: *12-channel respirometer for automatic biodegradation measurements*

Sample preparation

Vermiculite is a natural mineral that has a flake like appearance in its raw form. Its structure contains water interlamellar layers which are subjected to the hydration and dehydration processes. The hydration properties are controlled by the interlayer cations Mg^{2+} and minor amounts of Ca^{2+}, Na^{+}, and K^{+}. Vermiculite is most used as a soil amendment medium for growing food and other plants. When placed in soil, vermiculite helps seeds to germinate and soil to retain water. It also promotes soil aeration, preventing it from settling and ensuring that air and water can circulate freely. Vermiculite can be left in the ground, where it will naturally biodegrade and break down over time. It also continues to release minerals, such as potassium and magnesium into the soil. Soil containing vermiculite can be used in composting and it is actively recommended in composting mixes that require drainage.

The main advantage of using vermiculite is because its nature does not vary so much in composition between different places. Additionally, it does not suffer from transport problems like compost, which can face import bans or biohazard labelling in different countries due to varying regulations. When using vermiculite, it is still necessary to add an eluent from the compost (Figure 4), which is the perfect inoculum for microorganisms.

Figure 4: *Compost (left) versus vermiculite (right)*

Respirogram

The result of the biodegradation test is a respirogram that represents the biodegradability percentage as a function of time. If we want to analyse the respirogram, we need to know where the data is hidden (Figure 5). The lag phase and plateau phase have no information. The main information on the sample degradation is given in degradation phase, where we can see biodegradation kinetics. Sometimes, a waiting period of several weeks is necessary for the degradation phase to begin, and during this time, predicting the course of biodegradation is not feasible. Moreover, different plastics have varying shapes, and to accurately predict biodegradation, it is essential to have at least half of the measurement data to estimate the endpoint (extrapolate) with a ±15% error margin. Understanding the characteristic shape of the respirogram is also necessary. By implementation of a large data base and AI, more accurate predictions of biodegradation can be achieved.

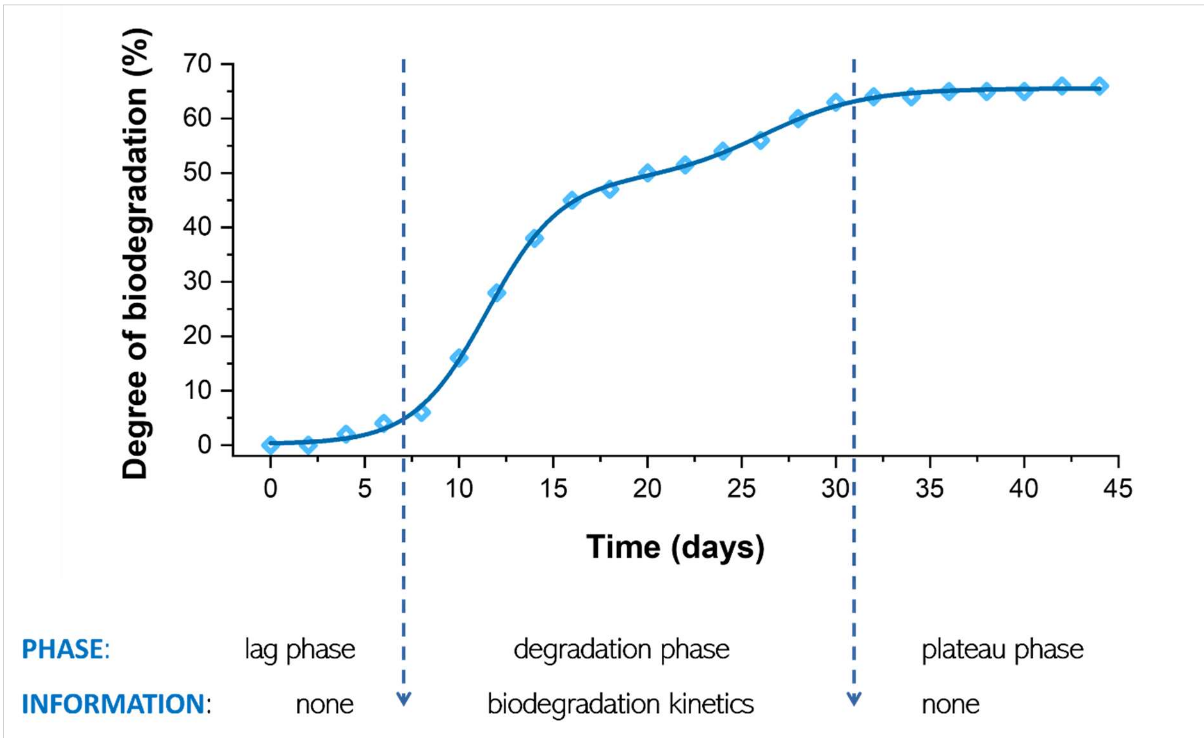

Figure 5: *Phases in the biodegradation process*

Similar results were observed in a comparative study using compost and vermiculite (Figure 6). Faster biodegradation rate was measured in the vermiculite/microcellulose sample mixture than in the compost/microcellulose mixture. At 54 days, the biodegradation of polylactic acid was faster in vermiculite than in compost, and after 110 days the difference in biodegradation was only 2 %.

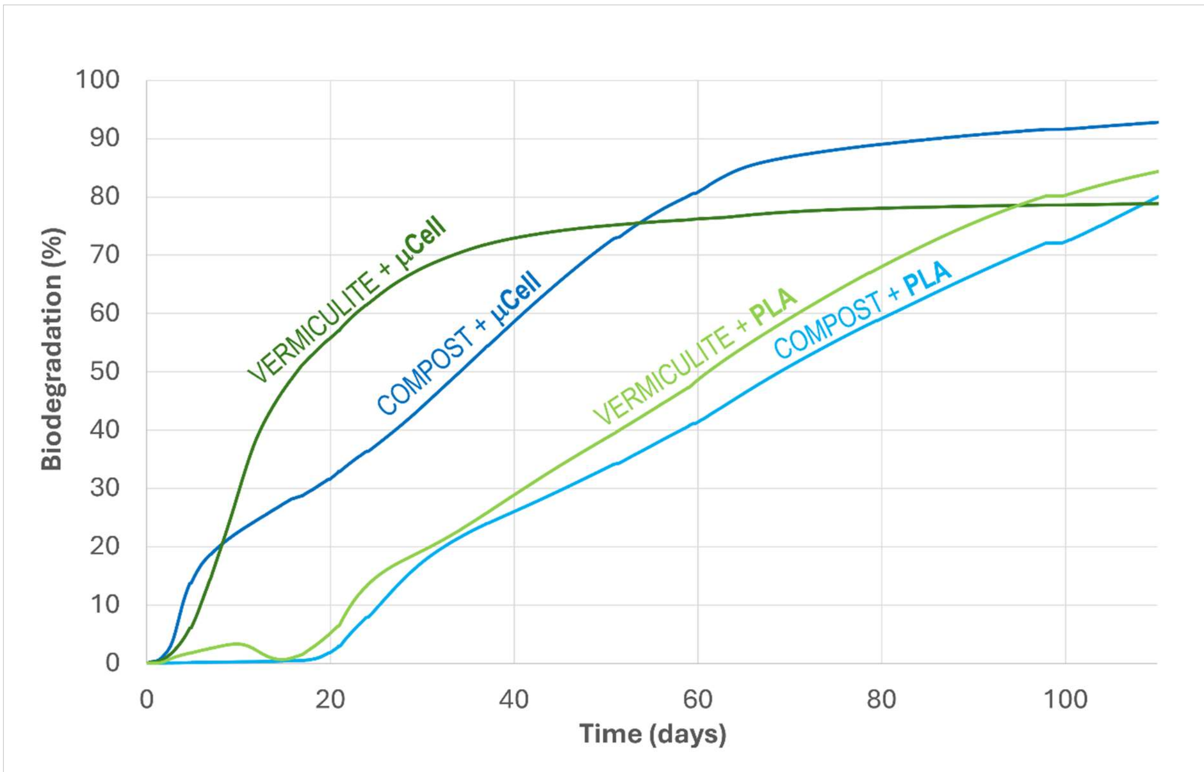

Figure 6: *Respirogram of polylactic acid (PLA) and microcellulose (µCell) recorded in compost and vermiculite.*

Table 1: Biodegradation of PLA and microcellulose in compost and vermiculite.

	Biodegradation at 54 days		**Biodegradation at 110 days**	
	µCell	PLA	µCell	PLA
Compost	75 %	38 %	92 %	82 %
Vermiculite	75 %	43 %	79 %	84 %

How to predict biodegradation

Each polymer has a distinct biodegradation pattern, reflected in the unique shape of the respirogram. This shape is influenced by the chemical and physical properties of the biodegradable polymer, the biological processes involved, and the mechanism of biopolymer breakdown.

To create a good biodegradation prediction model, we need to know what the shape of the respirogram is for a characteristic biopolymer or a related group of polymers. Based on this model, which illustrates the entire biodegradation process from start to finish, we can formulate a mathematical model that simulates the respirogram. In this respect it is crucial to identify where the relevant biodegradation lies. The initial and final segments offer minimal insight (Figure 5), while only the kinetic portion of the curve reveals the rate of biodegradation and helps predict its completion. The more data points we gather, the better we can predict biodegradation and the final extent of biological degradation. Without conducting initial one-time measurements to capture the biological degradation profile, it is impossible to make reliable predictions with a certain realistic margin of error. According to our experience, at least 50 % of the measurements are necessary to estimate the final degradation level with a margin of error of 10-15 %. This level of accuracy is sufficient for conducting rapid tests within a specific group of polymers. By employing such mathematical approach, we can anticipate biodegradation across any measured process and standard technique.

Other possibilities to enhance biodegradation rates and reduce analysis time include:
1) Utilizing powdered samples, which increase the surface area available for biodegradation, leading to faster break-down. Larger sample particles require more time since they must first disintegrate before biodegradation can commence.
2) Employing a highly active biodegradation medium. We can tailor this medium to suit our specific bioplastic type by feeding it with only one kind of plastic, followed by inoculating a fresh medium with selective microorganisms that effectively degrade our chosen plastic. For each specific type of bioplastic, it is essential to cultivate a dedicated culture adapted to that material.

Summary

Vermiculite can be utilized for biodegradation measurements with greater accuracy and improved reproducibility compared to compost. Standardizing the type of vermiculite and sample preparation for analytical laboratories can help establish a uniform biodegradation procedure. Such approach ensures greater reproducibility between laboratories that may otherwise be influenced by different compost conditions, such as local flora, fauna, and climate variations. By selecting appropriate mathematical procedures, we can also create a model for a specific biopolymer to predict the percentage of biodegradation before the measurement is complete. These approaches are helpful for conducting faster and accurate biodegradation tests.