APPLICATION NOTE

E-RES-003-2022/A1

Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in Soil with Respirometric Sensor System

Reference: **ISO 17556:2019** Plastics - Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved.

Tested with **RESPIROMETRIC Sensor System for plastic biodegradability code SA102A0166**

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.
VELP Scientifica, Italy Tel: +39 039 628 811 Fax: +39 039 628 8120 www.velp.com

APPLICATION NOTE

E-RES-003-2022/A1

Introduction

The analysis is used to obtain the optimal rate of plastic material biodegradation in test soil by controlling soil moisture, as well as, determining the ultimate biodegradability of the material itself. A non-specific soil is used as the inoculum because the test simulates the processes of biodegradation that occurs in a natural environment.

It is generally applied to the following materials:

- natural and/or synthetic polymers, copolymers or their blends;
- plastics that contain additives such as plasticizers or dyes;
- water-soluble polymers.

The plastic material is mixed with the soil. The mixture rests in a container for a period of time during which the amount of oxygen consumed (BOD) is determined or the amount of carbon dioxide is developed. The carbon dioxide is consumed in a special absorbent compound located in the headspace of the container. Oxygen consumption is determined by measuring the pressure difference inside the vessel, detected thanks to the **RESPIROMETRIC Sensor**.

The level of biodegradation, expressed as a percentage, is determined by comparing the BOD with the theoretical oxygen demand (ThOD), or by comparing the amount of carbon dioxide evolved with its theoretical amount (ThCO2). In the absence of this data, the biodegradability is determined for comparison with a material of known biodegradability or non-biodegradability taken as a reference.

For a further check, the the chemical oxygen demand (COD) of the material is performed to compare this value with the oxygen consumption at the end of the test.

The test result is the maximum level of biodegradation detected in the plateau phase in the biodegradation curve.

For the purposes of applying the standard used, the following definitions apply:

1. **Lag phase**: the time, measured in days, that elapses from the start of the test until the adaptation or selection of microorganisms and the degree of biodegradation are achieved of a chemical compound or organic substance reached is about 10% of the maximum level of biodegradation.
2. **Biodegradation phase**: the time, measured in days, from the end of the lag phase of a test, up to about 90% of the maximum biodegradation level reached.
3. **Plateau phase**: the time, measured in days, from the end of the biodegradation phase up to the end of the test.

The duration of these phases is variable and strongly depends on the characteristics of the material to be used for the biodegradation.

Sample

Garden universal soil
Mater-Bi (reference material)
Microcristalline cellulose 50 µm (reference material)

Reagents required

Absorption of carbon dioxide:

- Potassium Hydroxide (KOH) in flakes, commercial grade or non-deliquescent soda lime, 1.0-1.7 mm granules.

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.
VELP Scientifica, Italy Tel: +39 039 628 811 Fax: +39 039 628 8120 www.velp.com

DETERMINATION OF SOIL RESPIRATION

Sample Preparation

The sample used is garden universal soil for plants with ready-to-use fertilizer effect and slow-release fertilizer. The soil is prepared with the desired moisture content following as a reference the ISO-16072 - Soil Quality standard. The following analysis is performed:

- measurement of the actual volume occupied by the soil (after adjustment of water content);
- measurement of the pH of the soil (which should be between 6 and 8);
- moisture content.

Incubation takes place in the dark at a temperature of 20°C ± 1°C. The following tests are set up:

- **Blank** (2 replicates): the tests were carried out on 100g of soil using CO2 absorber. The pH of the solution was corrected to a value between 6.0 and 8.0;
- **Mater-Bi** as reference material (2 replicates): to the same quantities of soil used for blanks, add 200 mg of this reference material; the material is cut in quadrangular pieces of about 1 cm per side.
- **Microcrystalline cellulose** as reference material (2 replicates): at the same quantities of soil used for the blanks, add 200 mg of this reference material.

Analysis Procedure

1. Set the incubator temperature to 20°C.
2. Connect the Wireless DataBox™ and fill in the RESPIROSoft™ software database.
3. Weigh 200 mg of reference material in a beaker and add the soil.
4. Introduce about 6 pellets of KOH into the alkali collector.
5. Screw the RESPIROMETRIC Sensors onto each bottle and tighten.
6. Place the system in the **VELP FOC** at the desired temperature.
7. Wait about 30 minutes to reach the test temperature and for developing the typical initial pressure increase due to the vapor pressure and heating the air of the flask. To remove this additional pressure, it is advisable, to vent the test bottles to atmospheric pressure (by briefly loosening the ring nut).
8. Press START and run the test for 160 days or until a complete sample degradation.

Calculation

For the calculation of the THOD, for cellulose refer to the chemical formula $(C_6H_{10}O_5)_n$, while for the mater-bi refer to the typical composition of this material formed by a crystalline phase consisting of amylose, complexing agent and an amorphous phase consisting of amylopectin.

Taking into account any change in pressure for each aeration carried out, for the conversion from pressure measured in oxygen consumption BOD expressed as mgO2, refer to the law of perfect gases, i.e.:

$$BOD = M(O_2) / (R*Tm) * (Vt-Vl) / Vl * \Delta p(O_2)$$

M(O2) Oxygen molecular weight (32000 mg/mol)

R Gas constant (83,144 l*mbar/mol*K)
Tm Test Temperature
Vt Volume of bottle (in ml)
Vl Sample volume (in ml)
Δp (O2) Change in Pressure (mbar - hPa).

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.
VELP Scientifica, Italy Tel: +39 039 628 811 Fax: +39 039 628 8120 www.velp.com

Results

After 155 days of testing, the samples completely biodegraded.

The Cellulose sample reached the plateau phase after about 60 days, while the Mater-bi after about 130 days.

Figure 1 shows the pressure trends and the relative periodic aerations in order to maintain the oxygen concentration higher than 0 mg/l in the bottles and allow the aerobic degradation.

Figure 2 shows the accumulated and re-scaled pressure trends from the aerations, while **Figure 3** shows the same curves without the oxygen consumption of the blank test (endogenous respiration).

Figure 4 and 5 show respectively the trend of total BOD and for mass of introduced substrate.

The 50% biodegradation rate is reached after 16 days for cellulose and after 66 days for Mater-bi (Figure 5). The final biodegradation level was 95% for both samples.

Sample	THOD	BOD				Percentage of the biodegradation			
		30 days	60 days	90 days	End test	30 days	60 days	90 days	End test
	mgO2/g	mgO2/g	mgO2/g	mgO2/g	mgO2/g	%	%	%	%
Mater-bi	1900	495	869	1350	1800	26	46	71	95
Cellulose	1190	987	1115	1120	1129	83	94	95	95

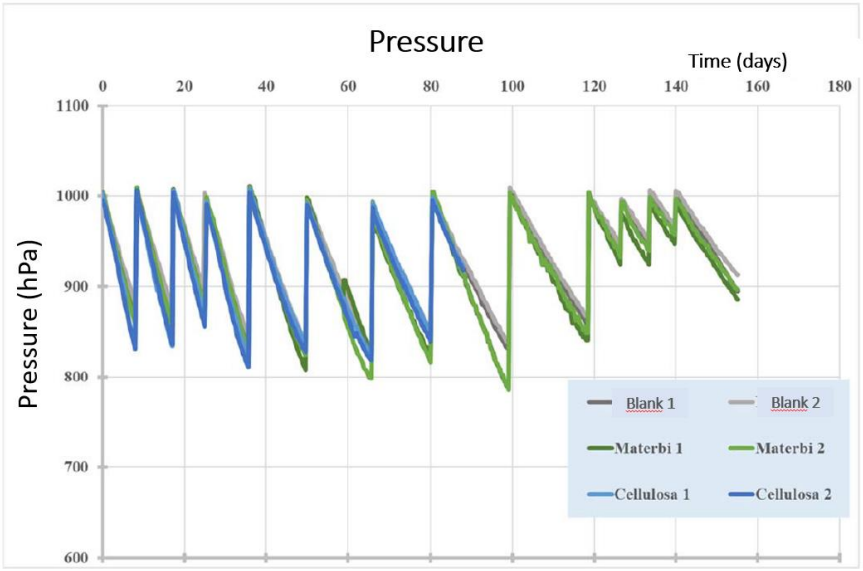

Figure 1: **Pressure graphs**. The discontinuities refer to the bottle re-aeration phases.

NOTE: when the pressure goes around 800mbar all the Oxygen inside the bottle is consumed. It is required to aerate the system (Unscrew the sensor and insufflate air inside the bottle).

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.

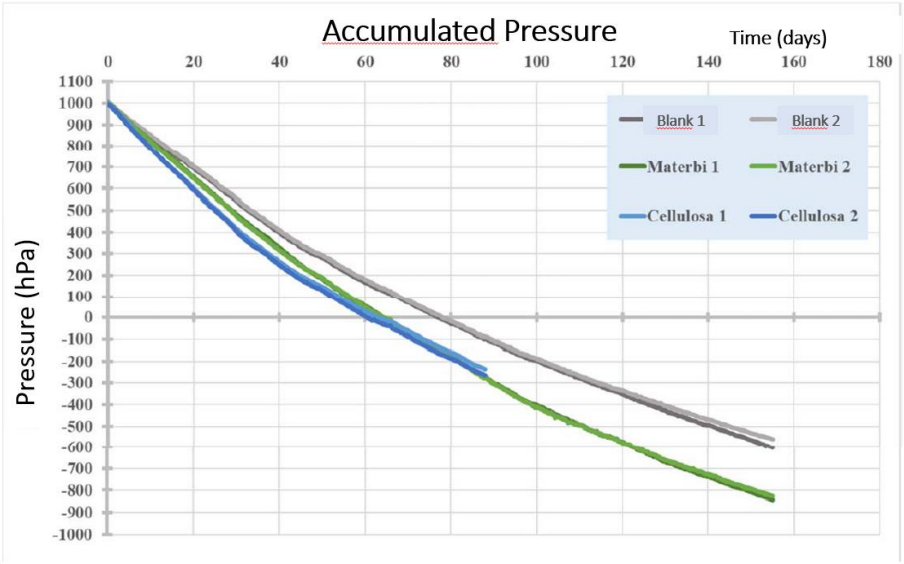

Figure 2: **Accumulated and re-scaled pressure trends**.

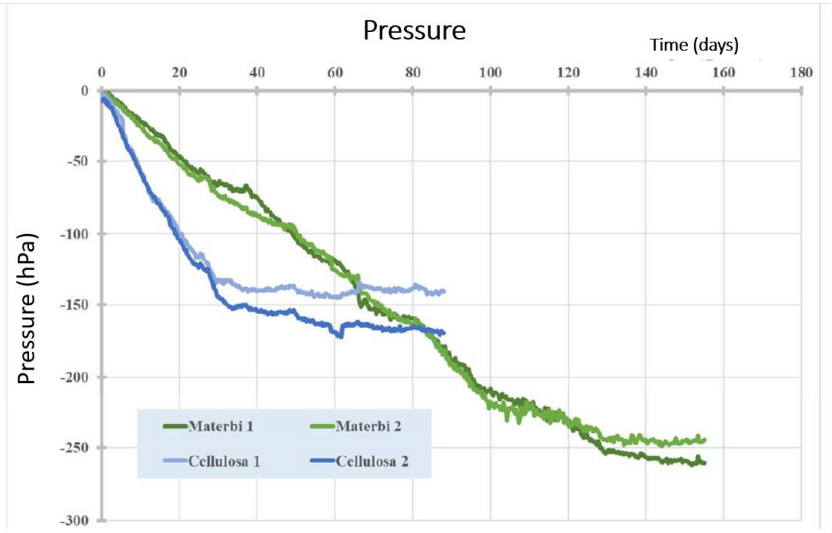

Figure 3: **Pressure graphs without blanks**.

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.
VELP Scientifica, Italy Tel: +39 039 628 811 Fax: +39 039 628 8120 www.velp.com

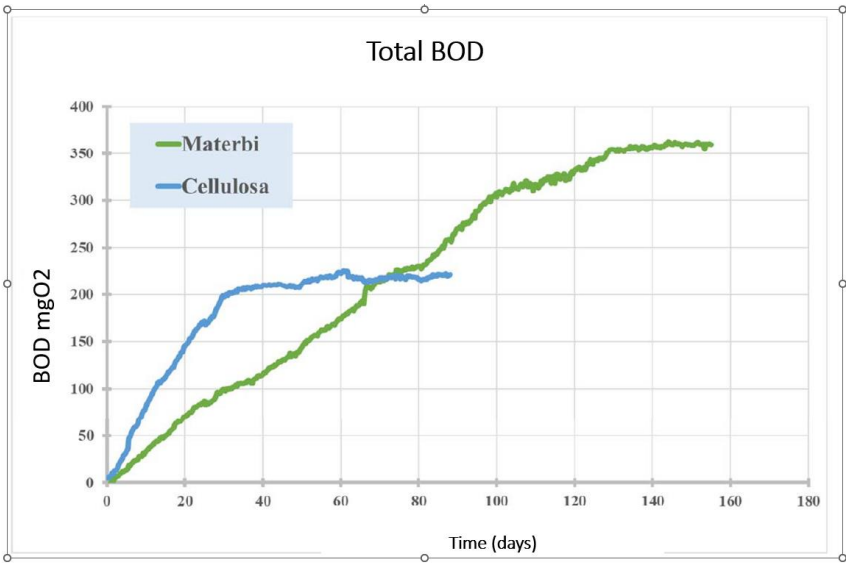

Figure 4: **Cumulative BOD trend (mg)**.

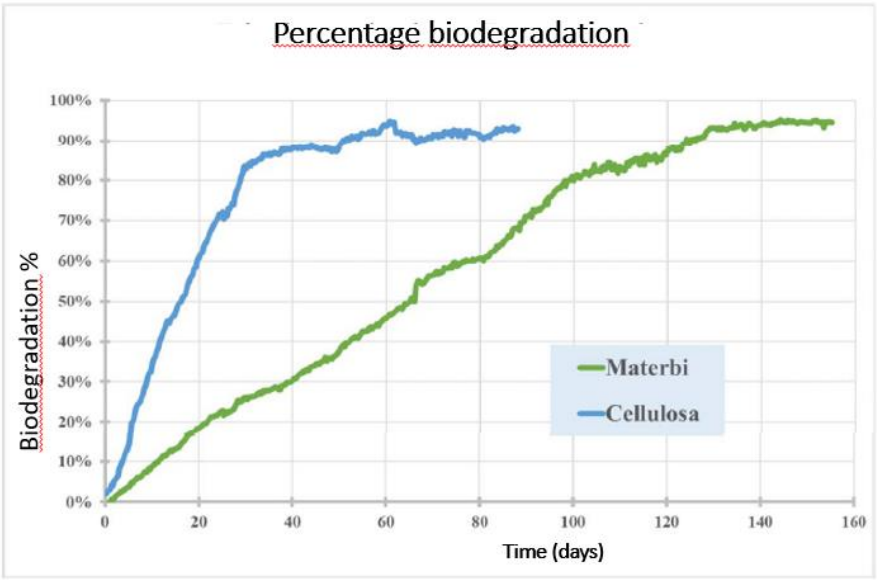

Figure 5: **Trend of the percentage of biodegradation**.

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.

Conclusions

RESPIROMETRIC Sensor System 6 for Plastic Biodegradability is a useful and reliable solution for the determination of ultimate aerobic biodegradability of plastic materials in soil according to the official norm ISO 17556:2019.

Thanks to the innovative wireless technology, the sensor transmits the pressure value to the Wireless Databox™, based on the data transmission frequency set before starting the analysis.

Results are then displayed from the intuitive **RESPIROSoft**™ the optimal solution for data management and comparison of the results.

Then, the intuitive interface optimally displays results for data management and result comparison.

Connect the RESPIROMETRIC Sensor to the exclusive **VELP Ermes Cloud Platform** to improve your laboratory experience.

Copyright © 2022 VELP Scientifica. All rights reserved.
No part of this publication may be reproduced or transmitted in any form or for any purpose without the express permission of VELP.
VELP Scientifica, Italy Tel: +39 039 628 811 Fax: +39 039 628 8120 www.velp.com