APPLICATION NOTE

E-RES-002-2022/A1

Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium with Respirometric Sensor System

Reference: **ISO 14851:2019** Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by measuring the oxygen demand in a closed respirometer.

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

VELP Scientifica, Italy Tel: +39 039 628 811 Fax: +39 039 628 8120 www.velp.com

DETERMINATION OF PLASTIC BIODEGRADABILITY IN AN AQUEOUS MEDIUM

Introduction

The analysis involves the use of aerobic microorganisms in an aqueous medium containing inorganic salts and the organic material to be tested (the only carbon source) with a concentration of organic carbon between 100 and 2000 mg/l. The mixture is stirred in a closed vessel.

Incubation takes place in the dark at an appropriate constant temperature, depending on the inoculum used for a period not exceeding 6 months. The developed carbon dioxide is consumed on a special absorbent compound located in the head space of the vessel. The consumption of oxygen is determined by the measurement of the pressure difference inside the container, detected via **VELP Respirometric Sensor**.

The level of biodegradation should be expressed as a percentage of the theoretical requirement of oxygen (ThOD) and of the material itself. In the absence of this data the biodegradability is determined for comparison with a material with that has known biodegradability or non-biodegradable, taken as a reference.

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

As another comparison method, the COD measurement can be performed.

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 in 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.

Samples

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.

Sample Preparation

The mater-Bi sample is cut into quadrangular pieces of about 1 cm per side.

The microcrystalline cellulose sample is analysed as is.

The inoculum is prepared from an activated sludge coming mainly from treatment plants of civil and industrial wastewater. Previously subjected to mixing and aeration, it is added to the solution at a rate of 5% (v/v). The 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 250 ml of standard test solution which is added inoculum and CO_2 absorber. The pH of the solution was corrected to a value between 7.2 and 8.0;

- **Mater-Bi** (2 replicates): to the same quantities used for blanks, add 2 g of this reference material;

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- **Microcrystalline cellulose** (2 replicates): at the same quantities used for the blanks, add 2 g 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 2 g of reference material in a beaker and add the inoculum.
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 180 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 and, omplexing 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.:

$$BOD = M(O2) / (R*Tm) * (Vt-Vl) / Vl * \Delta p(O2)$$

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)

Results

After 180 days of testing, the samples are completely biodegraded and have reached the plateau.

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).

The cumulative BOD curve (**Figure 4**) shows a first phase of slow degradation (0-100 days) followed by a more accelerated phase between 100th and 150th day for cellulose and between 100th - 180th day for mater-bi. The plateau is reached for both samples starting from the 180th day. The percentage of biodegradation of 50% is reached after 114 days (Figure 5), while on the 180th day for both the samples the biodegradation reaches 90%.

Sample	ThOD	BOD		Percentage of the biodegradation	
		180 days	End test	180 days	End test
	mgO2/g	mgO2/g	mgO2/g	%	%
Mater-bi	1900	1720	1854	90.5%	97.6%
Cellulose	1190	1141	1180	95.9%	99.2%

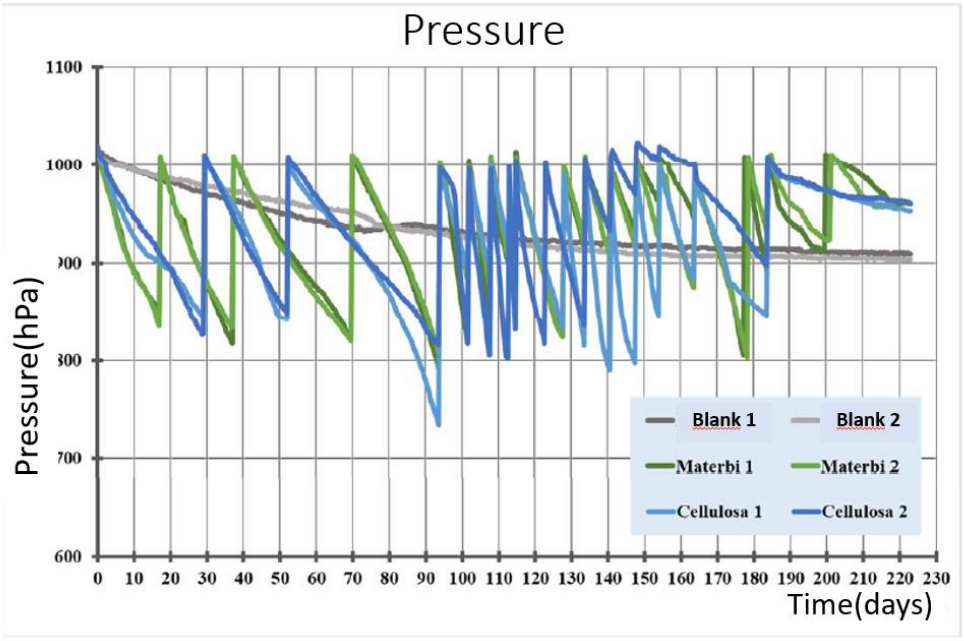

Figure 1: Pressure graphs. The discontinuities refer to the bottle re-aeration phases.

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

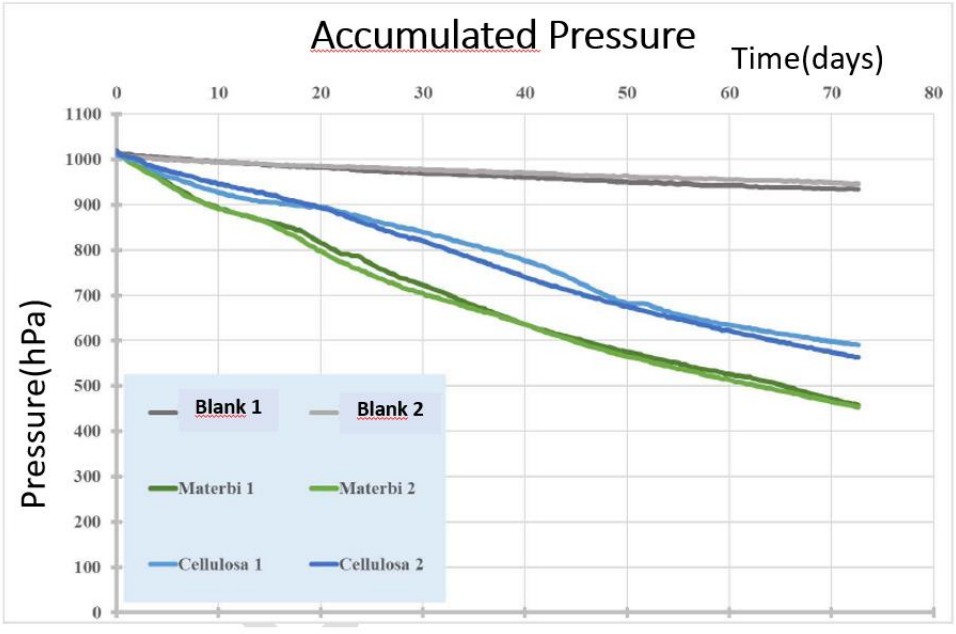

Figure 2: accumulated and re-scaled pressure trends.

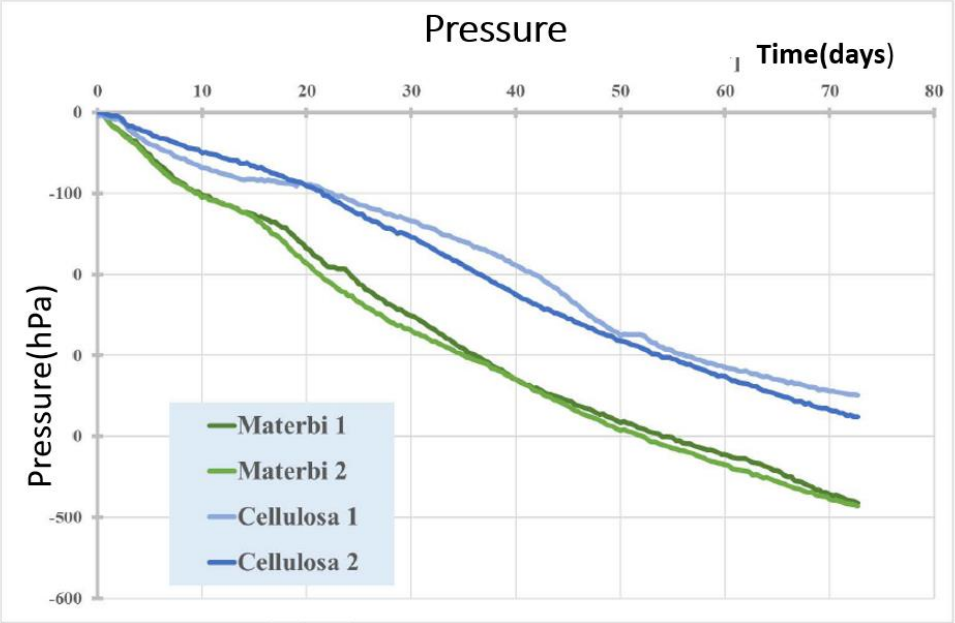

Figure 3: Pressure graphs without blanks

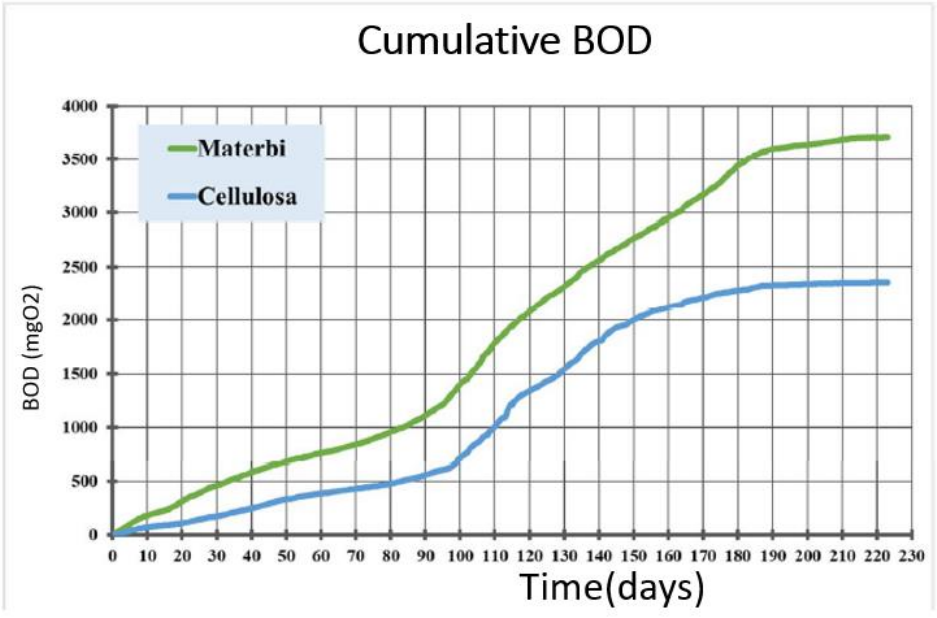

Figure 4: Cumulative BOD trend (mg)

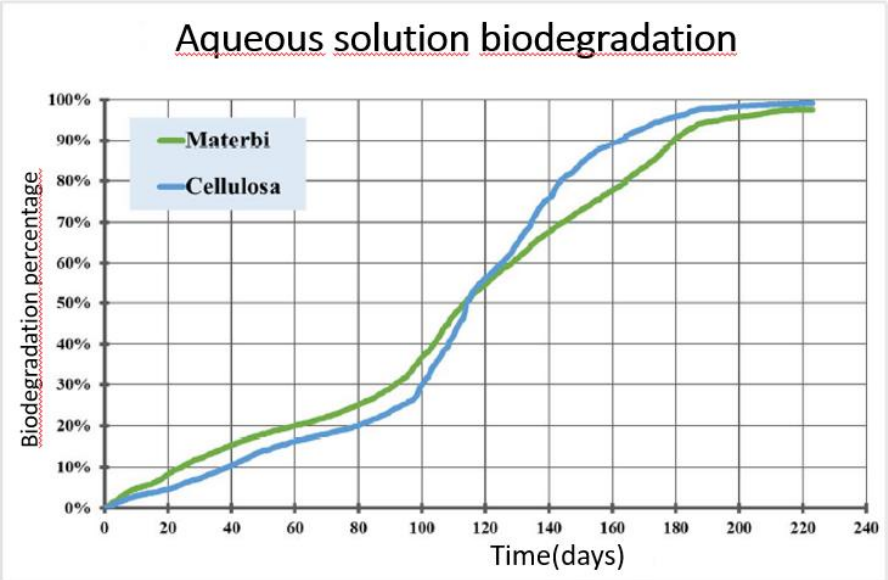

Figure 5: Trend of the percentage of biodegradation

Conclusions

RESPIROMETRIC Sensor System 6 for Plastic Biodegradability is useful and reliable solution for the determination of ultimate aerobic biodegradability of plastic materials in an aqueous medium according to the official norm ISO 14851:2019(E).

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.

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