H2020-NMBP-HUBS-2019

FlexFunction2Sustain

Open Innovation Ecosystem for Sustainable Nano-functionalized Flexible Plastic and Paper Surfaces and Membranes

Starting date of the project: 01/04/2020
Duration: 48 months

= Deliverable D3.4 =

Upgraded BIODEGRADIX line for verifying home compostability; and marine degradability ; Accreditation by TÜV Austria received

Dissemination level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Executive Summary

The FlexFunction2Sustain project aims to develop a network of upgraded labs, with the aim of studying biodegradation in different environmental media. To date, complete biodegradation studies (quality, biodegradation, disintegration, and ecotoxicity) are carried out within the laboratory, for domestic compost, industrial compost, and the marine environment. This is part of the "Circularity by design" services offered through the FlexFunction2Sustain Open Innovation Test Bed (OITB).

This Deliverable 3.4 (D3.4) covers developments made to the BIODEGRADIX pilot biodegradation installation available at IPC; as shown in Figure 1.

The upgrades described in this deliverable focus on:

- The installation of an elemental analyzer, to estimate the carbon, nitrogen, and hydrogen content of a material
- The installation of respirometers for the different environments studied (marine environment, domestic compost, and industrial compost)
- The installation of a disintegration tank allows the monitoring of oxygen and temperature
- The installation of a climatic chamber allows the regulation of temperature, light, and humidity.

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1. Introduction

This deliverable addresses the upgrade of the biodegradation line installation (BIODEGRADIX), allowing a complete biodegradation study within the framework of a TÜV accreditation. The improvements made possible by the project are the implementation of ecotoxicity tests in compost and the marine environment, as well as the implementation of the disintegration of compost on a laboratory scale.

Task 3.4 aims to test the biodegradability of new materials in different media. The standards used to demonstrate the biodegradation of materials will be those recognized in Europe, the specification standard for industrial compost (EN ISO 13432), for domestic compost (NF T 51800 or according to the TÜV AUSTRIA specifications), for the environment marine (ASTM D 6691 and TÜV AUSTRIA specifications). A complete biodegradation study according to the standards includes:

- A study of the quality of the plastic (presence of heavy metals, molecules toxic to the environment)
- A mineralization study (transformation of at least 90% of the organic carbon of the plastic into mineral carbon CO2) within a time set by the standards.
- A disintegration study on a pilot scale (ISO 16929) or laboratory scale (ISO 20200 or TÜV specifications), or 90% of the plastic must have disintegrated into pieces smaller than 2mm.
- An ecotoxicity study to ensure that there is no impact on the organisms living in this environment (Daphnia magna or plant depending on the targeted environment).

The biodegradation tests were conducted according to the specifications of the TÜV Austria organization. IPC has requested certification of the BIODEGRADIX biodegradation line by the TÜV AUSTRIA organization for the tests carried out in industrial compost, domestic compost and the marine environment.

As part of task 3.4 of FleFunction2Sustain, which aims to set up an OITB, with INL, IPC was able to test different types of materials to test their biodegradability in industrial and domestic compost.

The present document will be publicly available, as the executive summary of the Remix recycling pilot facility available at IPC and offered through FlexFunction2Sustain.

2. Results and discussion

2.1. Presentation of the biodegradation line

2.1.1. Polymer quality analysis

FTIR Microscopy & ATR

The infrared microscope as well as the ATR (attenuated total reflectance), makes it possible to obtain a profile of the plastic studied which allows accurate identification of the material.

This study is essential to accurately identify the plastics to be studied during the study. This profile can be used as a reference if the formulation of the material studied is changed.

Figure 1: Spotlight 400 FT-IR Imaging System, allowing to obtain an infrared profile of the plastics studied.

2.1.2. Biodegradation

2.1.2.1. Elementar Unicube

This elemental analyzer allows the simultaneous analysis of carbon, hydrogen, nitrogen, and sulfur in solid and/or liquid samples ranging from 0.1 mg to 1 g of material.
This device is dedicated to estimating the biodegradation of plastic. It allows knowing the proportion of carbon that plastic contains. This proportion will then be essential to know the theoretical content of carbon dioxide ($ThCO_2$) released by the test material.

Figure 2: Elemental analyzer allowing the measurement of carbon in polymers or plastics

This $ThCO_2$ is calculated as follows:

$$\mathrm{ThCO_2} = m \times w_C \times \frac{44}{12}$$

m : is the mass of the test material, in grams, placed in the test container;

w_c : is the carbon content of the test material, determined from the chemical formula or elemental analysis, expressed as a mass fraction;

44/12 : are the molecular and atomic masses of carbon dioxide and carbon, respectively

This result will then make it possible to calculate the percentage of degradation because it will be compared to the quantity of CO2 actually released over time from the materials tested.

2.1.2.2. Respirometer Echo

Echo respirometers make it possible to monitor the release of CO2 by bacteria in the environment under study. These devices are designed to monitor the concentration of oxygen and carbon dioxide (CO2) in the air in real time.

This system makes it possible to study the respiration of the environment by bringing constantly renewed air to the vessels. This has the advantage of having no stagnation of CO2 in the head space and therefore of having an air similar to that of the environment studied in reality (21% oxygen and 0.03% CO2).

This respirometer makes it possible to follow the analysis and test standard ISO 14855-1 which is included in the specification standards EN 13432 and NF-T 51800. It also makes it possible to follow the analysis and test standard ASTM D6691, used to study respiration in the marine environment.

The respirometer, using an air pump, imports ambient air under pressure. This air is analyzed on entry before being brought to the incubators. The air penetrating into the vessels will balance the gases and take charge of CO2, due to the respiration of the bacteria in the environment. Then this charged air will be analyzed by the device (infrared analyzer) and by deducing the concentration of CO2 initially present in the air, we have the concentration of CO2 actually released by the bacteria in the environment at each analysis time.

Then the quantity of CO2 emitted is converted into percentage of biodegradation according to the following formula :

$$D_t = \frac{(CO_2)_T - (CO_2)_B}{ThCO_2} \times 100$$

- **$(CO2)_T$** is the cumulative amount of carbon dioxide evolved in each composting vessel containing test material, in grams per vessel
- **$(CO2)_B$** is the mean cumulative amount of carbon dioxide evolved in the blank vessels, in grams per vessel
- **$ThCO_2$** is the theoretical amount of carbon dioxide which can be produced by the test material, in grams per vessel.

To increase the number of biodegradation experiments, IPC has invested in 3 respirometers:
- 2 of 12 channels
- 1 of 36 channels

This allows having 48 samples in parallel. Knowing that the samples in biodegradation must be studied in triplicate to have good repeatability, good to be considered statistically valid.

Figure 3: Respirometer allows the monitoring of the release of CO2 over time in the compost or the marine environment

2.1.3. Disintegration

The disintegration study is an integral part of the specification standard for industrial (EN 13432) and domestic (NF T 51800) compost. This step makes it possible to ensure that the product in its final form has a thickness allowing its complete fragmentation (> 90%).

The standards require that fragmentation of less than 2 mm be observed. There are two types of disintegration, the first at the laboratory scale and the second at the pilot scale.

2.1.3.1. Laboratory scale disintegration (ISO 20200)

The study of disintegration on a laboratory scale is carried out according to the ISO 20200 standard. This standard imposes a study of disintegration in tanks having dimensions of 30 cm x 20 cm x 10 cm.

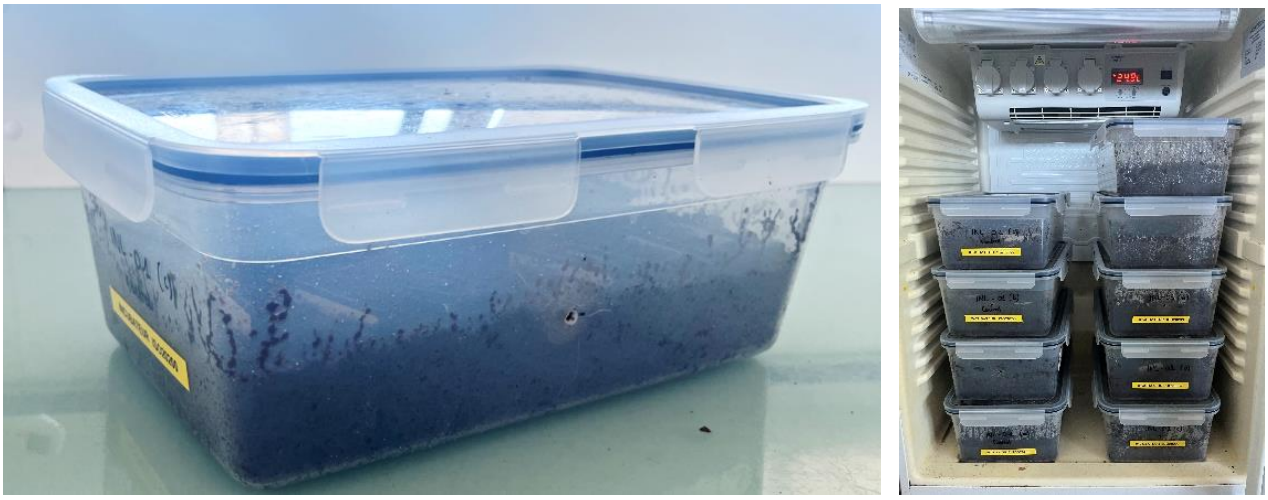


Figure 4: Incubator for the study of disintegration on a laboratory scale

The bio-waste used to study the composting process is also standardized:

- Sawdust
- Rabbit food
- Mature compost
- Corn starch
- Sucrose
- Corn oil
- Urea

This standard makes it possible to study the disintegration of parts in the form of a finished product or in the form of a film, requiring only a small quantity of plastic (about 500 g). The study of disintegration in domestic compost is carried out at 25°C for a maximum of 6 months. The study of disintegration in industrial compost is carried out at 58°C for a maximum of 6 months.

Bio-waste not being in sufficient quantity to increase the temperature and mimic the industrial composting process, the entire bin is placed in an enclosure to imitate the rise in temperature. Thus for industrial compost, the thermophilic phase at 58°C lasts for a maximum of 90 days, and then a mesophilic phase at 25°C can also begin for a maximum of 90 days. At the end of this study, the disintegration is judged after having sieved the compost of 2 mm and isolated the pieces of plastic retained by the sieve. We must not find more than 10% of the weight initially incorporated, to consider the plastic as biodegradable.

2.1.3.2. Pilot scale disintegration (ISO 16929)

The study of biodegradation on a pilot scale makes it possible to work with a larger quantity of bio-waste (approximately 35 kg of wet matter). This method has the advantage of being able to prepare the compost for future ecotoxicity tests on two types of plants. The disintegration test is carried out under defined and standardized composting conditions on a pilot scale.

The test material is mixed with fresh biological waste at a precise concentration and introduced into a defined composting environment (temperature, humidity, pH). A ubiquity natural microbial population spontaneously begins the composting process and the temperature rises. This bio-waste is regularly turned over and moistened throughout the study. The temperature, the pH value, the content of Humidity and gas composition are regularly monitored.

Figure 5: Decay vessel used for pilot scale standard. Tracks home and industrial composting

The composting process is maintained until a fully stabilized compost is obtained, which is generally the case after 12 weeks. The compost is observed visually once a week and observation of the disintegration of the material is carried out. At the end of the test, the maturity of the compost is determined and the mixture of compost and test material is sieved using sieves with 2 mm and 10 mm mesh. The disintegration of the test material is evaluated on the basis of the pieces of plastic recovered greater than 2 mm. The compost obtained at the end of the composting process can be used to carry out the ecotoxicity test imposed by the standards for domestic compost and industrial compost.

We work at IPC in large 100 L tanks allowing us to have the minimum volume of bio-waste required in the standard (35 L). These tanks are made of stainless steel, heat-resistant and non-biodegradable.

The standard imposes precise temperature kinetics for the monitoring of biodegradation in industrial compost. This kinetics is controlled using an electric blanket. Our system (echo supplier) makes it possible to follow throughout the process the core temperature of the compost as well as the oxygenation of the compost. These measurements are automated, which allows regular monitoring throughout the 12 weeks of incubation.

This method, therefore, requires working with larger quantities of plastics, approximately 1 kg in the form of a final product for a disintegration test and 6 kg in the form of a powder if an ecotoxicity test is planned afterwards.

The tested plastic will be considered disintegrable if 90% of its initially incorporated weight has disintegrated into pieces smaller than 2 mm.

2.1.3.3. Disintegration in the marine environment (according to TÜV specifications)

Disintegration in the marine environment has no specific standard and in Europe, no standard is recognized for testing this parameter.

As a result, IPC bases itself on the specifications of TÜV Austria to study this parameter. Marine disintegration is carried out at 30°C in natural seawater for a maximum of 84 days. Incubation takes place in a thermostatically controlled enclosure allowing seawater to be stirred at 170 rpm.

Figure 6 : Thermostatically controlled enclosure allowing agitation for studying the marine environment

This disintegration test imposes the same conditions of use as the ASTM 6691 standard. Plastic will be considered disintegrable if we do not find more than 10% of pieces larger than 2 mm.

2.1.4. Ecotoxicity

2.1.4.1 Plant ecotoxicity (domestic and industrial compost)

The ecotoxicity test in domestic and industrial compost is carried out according to OECD 208 (Terrestrial plant test: test for emergence of seedlings and growth of seedlings). This test makes it possible to study the acute toxic effect on two types of plants (monocotyledon and dicot). The test consists of bringing the seeds and the compost having received the plastic to be tested into contact. After 21 days of observations, the plants are photographed and recovered. The size of the plants, the production of biomass (wet and dry), as well as the number of germinated plants, are examined and compared to the control compost (without plastic). For a plastic to be considered non-toxic to plants, at least 90% of the seeds must be germinated compared to the control, and biomass production of 90% compared to the control. No phytotoxic effects should be observed on plants.

This study is carried out in an enclosure with controlled temperature, humidity, and lights throughout the study. To carry out this test, IPC invested in a Memmert HPP 750 eco climatic chamber allowing the control of all the parameters.


Figure 7: The climatic chamber allows the incubation of plants with controlled humidity, temperature and light.

2.1.4.2. Marine ecotoxicity

At IPC we test ecotoxicity in the marine environment following the recommendations of the OECD 202 and the specifications of TÜV Austria. The principle is to test after the plastic has biodegraded in the environment, the impact of biodegradation products on Daphnia magna.

Young daphnids, less than 24 hours old at the start of the test, are exposed to water contaminated with biodegradation products during the incubation process. Immobilization is recorded at 24 and 48 hours, then compared to control values (pure water). The results are analyzed and for a plastic to be considered non-toxic, at least 90% of Daphnia magna must survive the test.

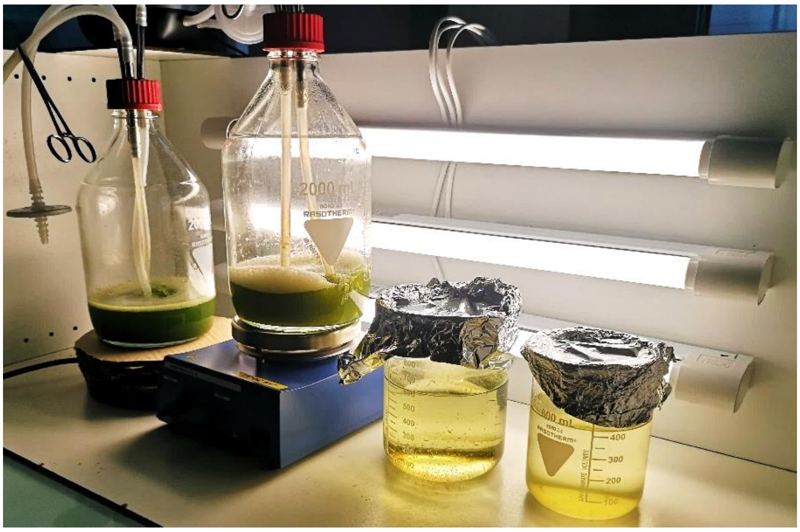



Figure 8: Presentation of the breeding of Daphnia magna at IPC used for the ecotoxicity test.

2.2. Results of the tests carried out within the framework of FF2S

The BIODEGRADIX pilot line has been tested with 3 of the use cases from the WP5 :

- **Hueck Folien (Task 5.1)**: Development of biodegradable optical structures (industrial compost).
- **Procter & Gamble (Task 5.2)** : Seawater degradable shampoo sachets (marine biodegradation)
- **Sonae (Task 5.5):** Paper-based fresh food package (home compost)

2.2.1. Hueck Folien (Industrial compost)

IPC has carried out several tests for this study:

- First trials with 6 different formulations which started on December 21, 2020, and ended on February 25, 2021
- Second trials with 4 different formulations which started on May 18, 2021, and ended on 30 October, 2021
- Third test with 2 different samples which started on July 5, 2022 and ended on October 17, 2022
- Fourth test with 3 different samples which started on November 29, 2022, and which is still ongoing.

All the tests were carried out under industrial composting conditions (58°C)

2.2.1.1. First test (December 2020)

In this experiment, we tested 6 formulations. The details we have on these formulations are as follows (Table 1).

Formulation	m-%		
#	C (g/Kg)	O	H
1	700	0,21	0,09
2	580	0,35	0,07
3	630	0,29	0,08
4	680	0,22	0,1
5	650	0,27	0,08
6	550	0,38	0,07

Table 1: Elementary analysis of the different formulations tested

The study was launched for 65 days. The results are shown in the graph in Figure 9.

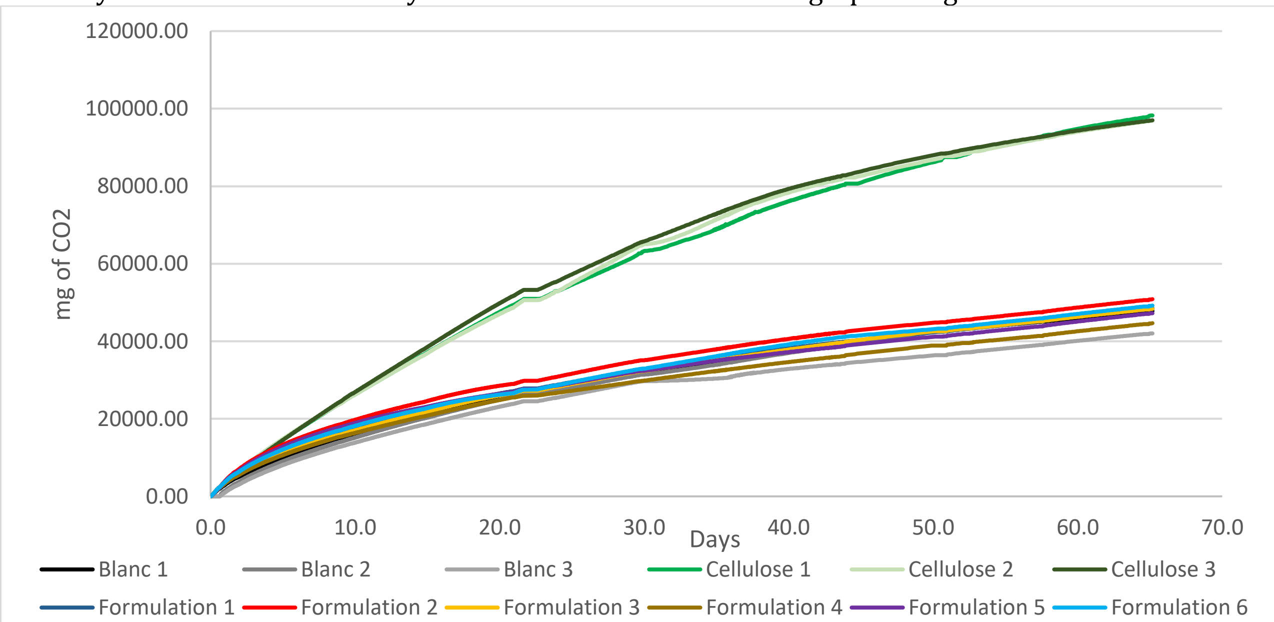


Figure 9 : CO_2 production over time during the industrial composting study

The study lasted 65 days. It made it possible to test 6 different formulations and our positive control (cellulose). The tests were not carried out in duplicate or triplicate. Only one sample per formulation was tested. What we have observed during these 65 days is that only our positive control had greater respiration than the blank (compost alone without adding carbon).

The blank produced 46058 mg of CO_2 after 65 days, and the cellulose produced twice as much CO_2 with 97408 mg of CO_2. All the formulations had production similar to that of white:

- Formulation 1: 48582 mg of CO_2
- Formulation 2: 50849 mg of CO_2
- Formulation 3: 48403 mg of CO_2
- Formulation 4: 44646 mg of CO_2
- Formulation 5: 47234 mg of CO_2
- Formulation 6: 49206 mg of CO_2

The production of CO_2 (mg) was translated into a percentage of biodegradation thanks to the value of organic carbon contained in the different formulations tested (Figure 10).

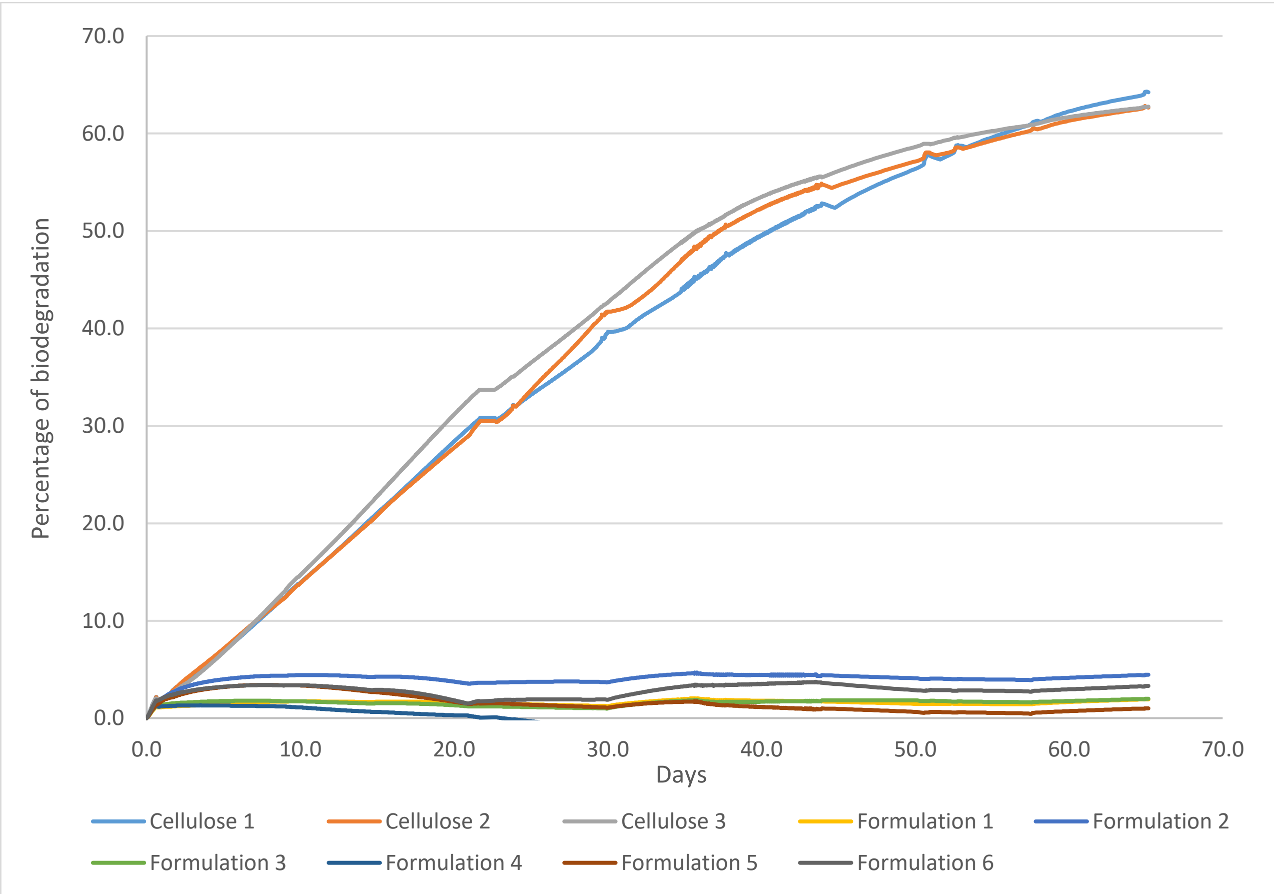


Figure 10 : Percentage of biodegradation in industrial compost over 65 study days

The percentage of biodegradation after 65 days is 63% for cellulose. All the other formulations have a percentage of biodegradation between 1.3% and 4.7%. The experiment was stopped after 65 days because all **the formulations showed no sign of biodegradation during this experiment**.

2.2.1.2. Second tests (May 2021)

In this test we tested 4 different formulations, the details of which are as follows:

- Formulation 1, carbon content of 0.59 g/g
- Formulation 2, carbon content of 0.65 g/g
- Formulation 3, carbon content of 0.7 g/g
- Formulation 4, carbon content of 0.58 g/g
- PLA (polylactic acid), 0.5g/g carbon content of 0.5 g/g

The experiment lasted 140 days, the 4 formulations were tested in duplicate as well as the PLA. The percentage of biodegradation was obtained by observing the production of CO_2 (mg) over time and subtracting the respiration of the compost alone (white) over time. The results are shown in Figure 11.

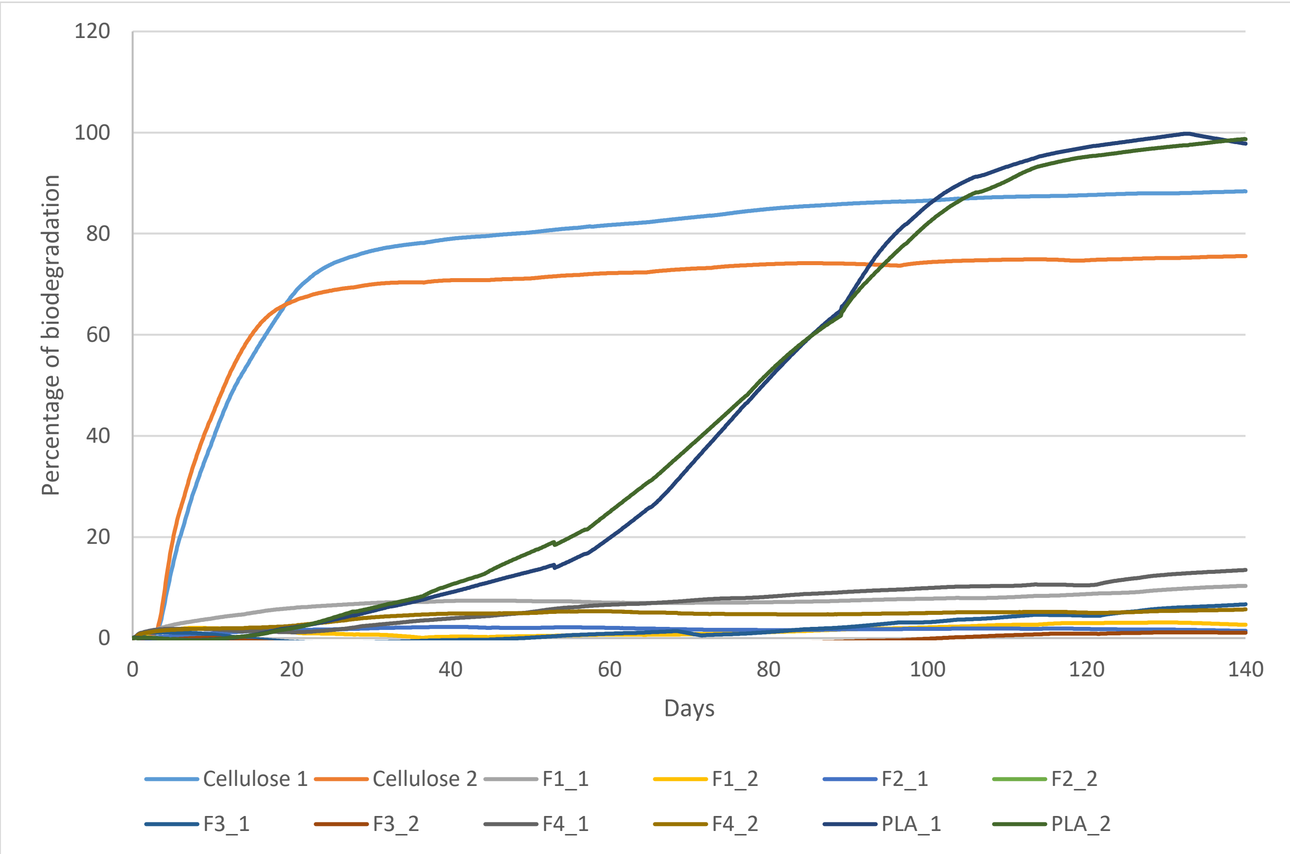


Figure 11: Percentage of biodegradation of the different samples over 140 days of studies in industrial compost

Our positive control for the experiment (cellulose) showed very rapid biodegradation kinetics from the start of incubation. Cellulose degraded exponentially reaching a plateau of 82% after 140 days.
The biodegradation study on PLA showed a significant latency phase (15 days), this latency phase is normal and well documented. There is a PLA hydrolysis time that is not visible when only the release of CO_2 is monitored. The biodegradation kinetics of PLA reached a plateau, after biodegradation of 98.3%.

The study of the biodegradation of the 4 formulations made it possible to demonstrate that no visible sign was observed during the 140 days of study. The percentage of biodegradation of all the formulations is between 0% (Formulation 2) and 6.49 %(Formulation 1). **This percentage does not allow the formulations tested to be defined as biodegradable in industrial compost.**

2.2.1.3. Third test (July 2022)

For this test, two samples were tested:

- Cellulose hydrate, containing 42.42% carbon (45 µm), 1 % of resin number 4
- PLA, containing 56.73% carbon

This experience lasts 90 days. The results are presented in Figures 12 and 13.

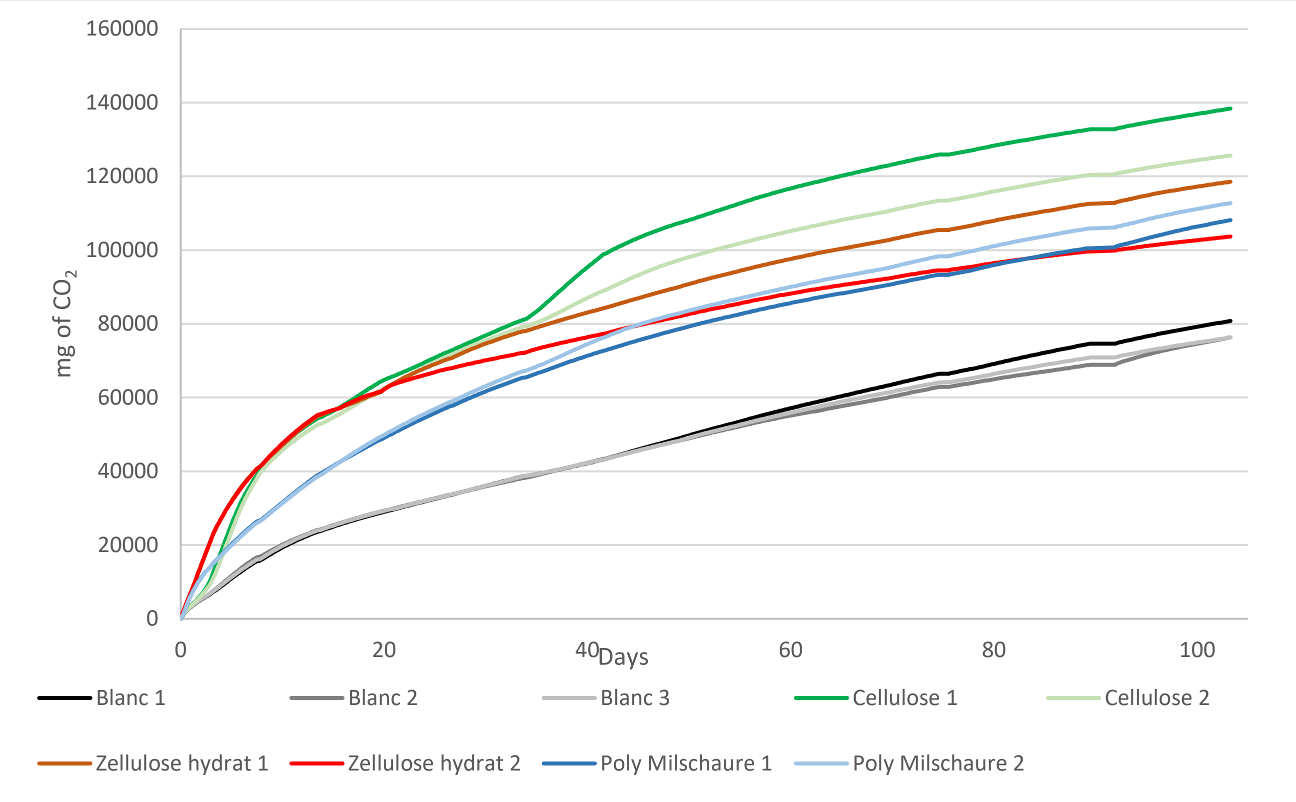


Figure 12 : Release of CO2 during the 103 days of incubation in industrial compost (Poly Milschaure stands for PLA)

The measurement of CO_2 release over time shows constant respiration of the white, with a maximum production of 78,300 mg ($^{+}/_{-}$ 2246 mg) of CO_2 after 103 days. The positive control of the experiment (IPC cellulose) shows a very rapid release of CO_2 in the first 15 days, then the kinetics of the release of CO_2 decreases. After 103 days, the IPC cellulose observes a release of 131,814 mg ($^{+}/_{-}$ 6356 mg) of CO2. The cellulose given by the company (Cellulose hydrate) shows CO_2 release kinetics similar to that of IPC cellulose for the first 25 days. Then this kinetic decreases and becomes similar to that of white. After 103 days, the cellulose hydrate showed a maximum release of CO_2 of 11,0938 ($^{+}/_{-}$ 7409 mg) mg of CO_2. The CO_2 release kinetics during the first 5 days of the experiment was so great that the values measured were within the quantification limit of the device. For 5 days the measured values are therefore perhaps underestimated.

The PLA sample tested showed constant CO_2 release kinetics over time until it reached the same dynamics as the blank. After 103 days, the sample has a release of 11,0194 ($^{+}/_{-}$ 2322) mg of CO_2

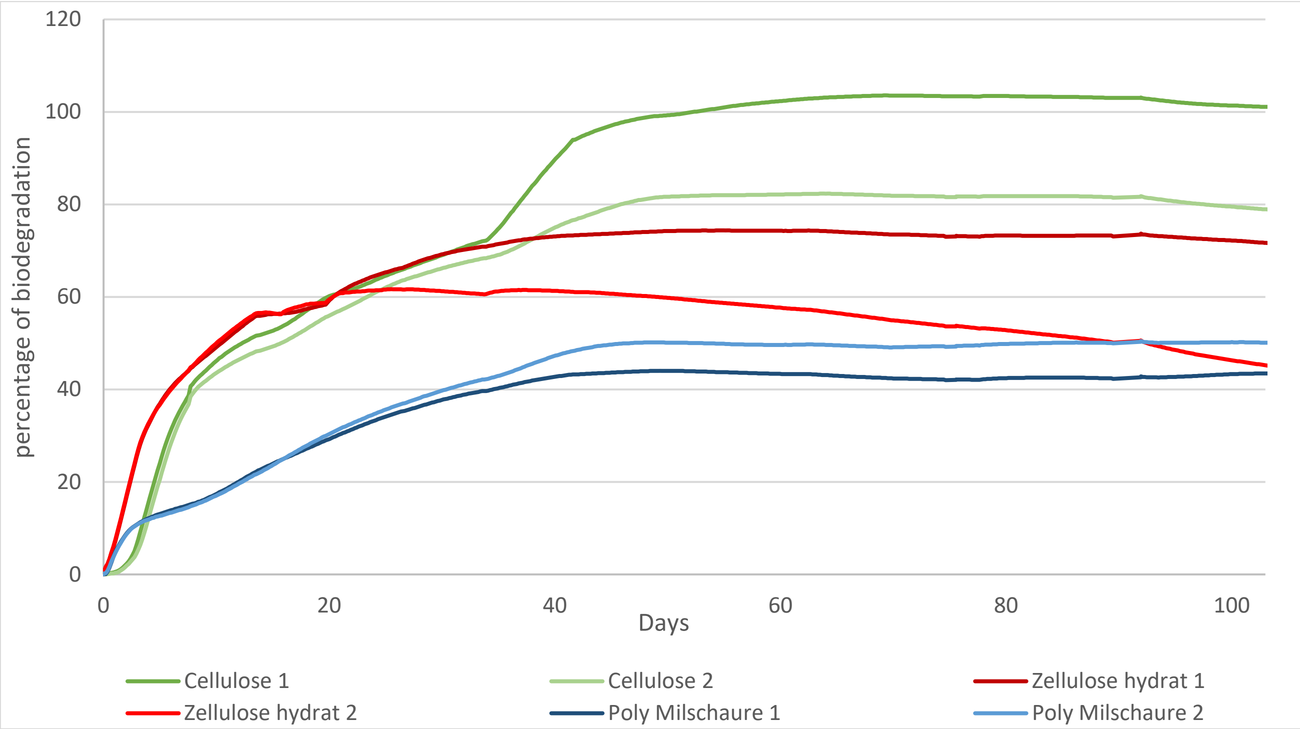


Figure 13 : Percentage of biodegradation over time of the different samples under industrial compost conditions (Poly Milschaure stands for PLA)

The release of CO2 is then converted into a percentage of biodegradation thanks to the proportion of organic carbon in the samples.

The study of biodegradation shows that for the positive sample of IPC (cellulose), the biodegradation is very rapid the first 10 days (reaches 45.3%), the kinetics of biodegradation then decelerates until the 40th day (reaches 81.7 %), reaching a plateau of 90% after 90 days of study.

The biodegradation study provided by the manufacturer shows very rapid biodegradation kinetics for the first 12 days (reaching 53.7%). The cellulose hydrate sample did not exhibit the same behavior in the two replicates. The first sample reached a plateau at 74% biodegradation while the other sample reached a maximum biodegradation percentage of 61%. Then the percentage of biodegradation has "decreased". This decrease is only a bias observed in the calculation of biodegradation. This means that from the moment the percentage of biodegradation decreases, the respiration observed in this sample is less important than the respiration observed in that of the control (blank).

The experiment should have stopped on the 50th day when all the samples had reached the plateau.

The study of the PLA sample shows almost constant biodegradation kinetics up to the 40th day and reaches a percentage of 67%. Then the kinetics reached a plateau at 68% before stagnating throughout the rest of the experiment.

In the context of this experiment, **we cannot consider the cellulose hydrate and the PLA sample as biodegradable in industrial compost according to our study parameters.** During the first 5 days of study, we were able to underestimate the biodegradation kinetics of the hydrated cellulose sample due to the quantification limit of the device.

2.2.1.4. 4th test (in progress)

During this test, we tested 3 different samples:

- Natureflex
- NIL Bioresin first generation
- NIL Bioresin 2nd generation

All of these tests were carried out in duplicate, this parameter having great importance on the value of the standard deviations.

The production of CO_2 is presented in figure 14.

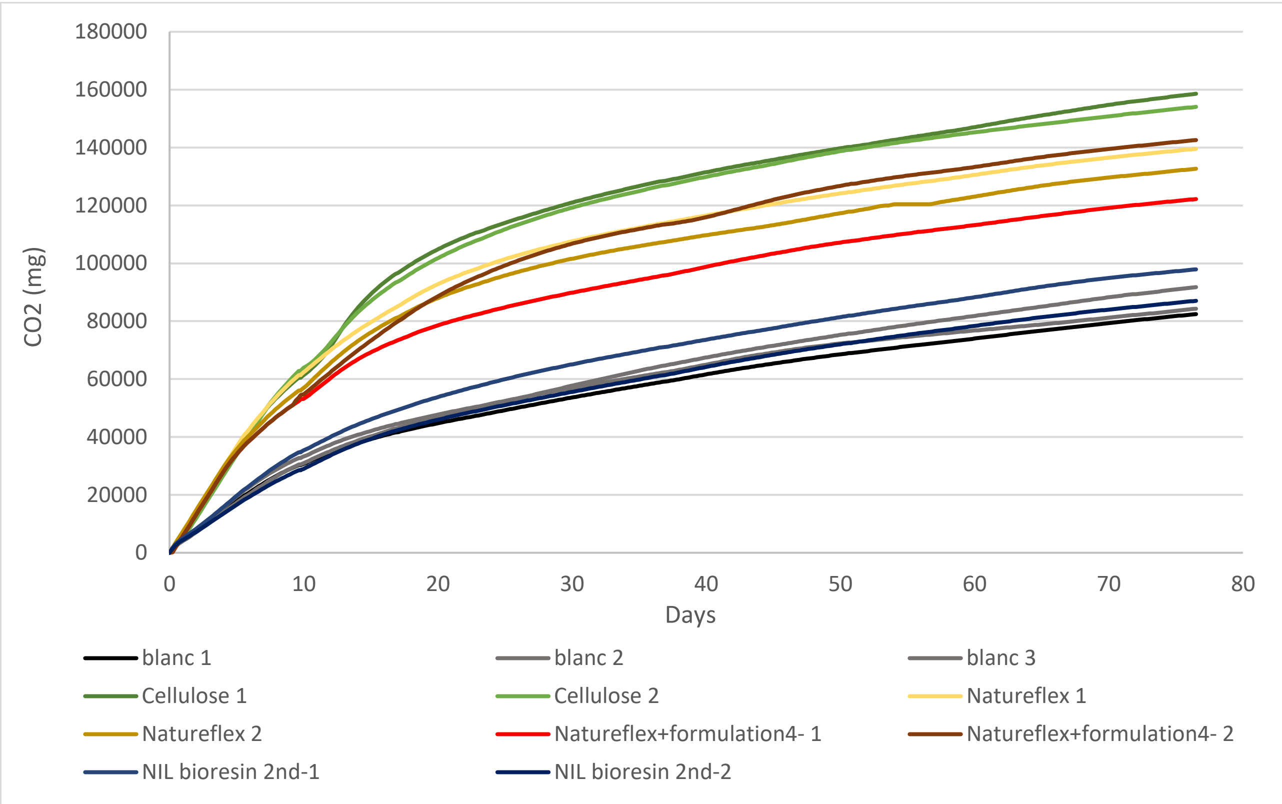


Figure 14 : CO2 production for the different samples after 76 days of testing in industrial compost

The white ("*blanc" on Figure 14*) shows a linear release of CO_2 over time for the first 15 days until reaching 40701 (+/- 1410) mg of CO2. Then the kinetics of CO2 production decreases to reach 85966 (+/- 4915) mg of CO2 after 76 days.

Our positive control of the cellulose experiment showed very significant CO2 release kinetics during the first 20 days to reach the value of 101,662 (+/- 3360) mg of CO2. The CO2 release kinetics continued to increase to reach 151,633 (+/- 8066) mg of CO2, i.e. a production 1.77 times greater than the blank.

The Natureflex sample shows very significant CO2 release kinetics for the first 20 days. Our respirometer reported respiration close to the limit of quantification during the first 5 days of the experiment. This can lead to an underestimation of biodegradation. Natureflex produces 135926 (+/- 4867) mg of CO2 after 76 days of experience, i.e. a production 1.58 times higher than that of white. The kinetics of CO2 now follows that of white (parallel curve) since the 30th day.

The 1st generation NIL bioresin sample showed CO2 production kinetics comparable to that of the Natureflex sample for the first 5 days. Just like the Natureflex sample, the production of CO_2 was very high and close to the quantification limit of the device. The CO_2 production kinetics then decreased to reach a plateau at 132,291 (+/- 14408) mg of CO_2, i.e. a production 1.53 times greater than that of the blank. It is important to note that the duplicates of this sample are very divergent which brings a significant standard deviation. Not having a third replicate to confirm the experiment, the average presented in this graph may not be representative of reality.

The 2nd generation NIL Bioresin sample showed similar kinetics to the blank, with CO2 production reaching 92286 (+/- 7686) mg, i.e. a production 1.07 times greater than that of the blank.

Biodegradation was studied by taking into account the respiration of the blank samples and the carbon content of each sample (figure 15).

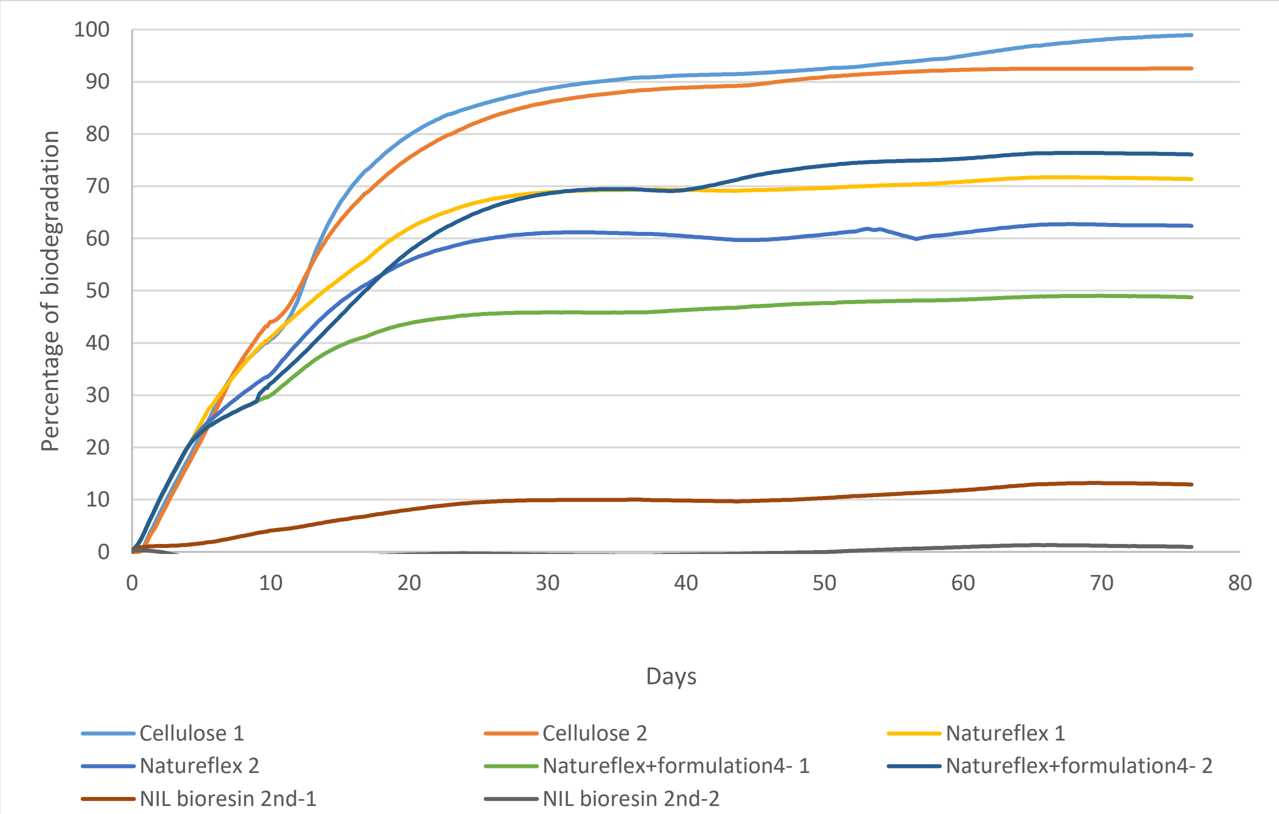


Figure 15 : Percentage of biodegradation after 76 days of study in industrial compost

Our positive control of the cellulose experiment shows linear biodegradation kinetics during the first 25 days, then stabilized forming a plateau at 89.71% (+/- 10%) of biodegradation. Our compost showed very high bacterial activity because the cellulose was 70% biodegraded in 18 days.

The Natureflex sample shows very significant biodegradation kinetics during the first 20 days and then stabilizes to reach a plateau from the 30th day. The observed biodegradation is 66.92 (+/- 6.33)%, which corresponds to 74.6% of the cellulose. To date, with our experimental parameters, the Natureflex sample cannot be considered biodegradable in industrial compost.

The NIL bioresin 1er sample shows very significant biodegradation kinetics during the first 20 days and then stabilizes to reach a plateau from the 30th day. The observed biodegradation is 62.45 (+/- 19.34) %, which corresponds to 69.61% of the cellulose. For this sample, we observe a significant standard deviation. This standard deviation is authorized by the standard (< 20%). However, the average may not represent reality. To date, with our experimental parameters, the NIL Bioresin 1er sample cannot be considered biodegradable in industrial compost.

The NIL bioreisn 2nd generation s showed no significant signs of biodegradation. The observed biodegradation is 6.95 (+/- 8.45)%. **To date, with our experimental parameters, the NIL bioresin 2nd generation sample cannot be considered biodegradable in industrial compost.**

2.2.2. Procter and Gamble (Marine environment)

The purpose of this study is to determine the degree of biodegradation of plastic materials in the marine environment according to the ASTM 6691 standard. The ability to biodegrade is evaluated in natural seawater, incubated at 30°C for 6 months at 170 rpm/minute. In this study we will carry out the study according to the standard: “ASTM6691-01-2017 Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum. »

The test material is mixed with natural seawater supplemented with 0.5 g.L-1 of NH4Cl and 0.1g.L-1 of KH2(PO4), making it possible to study the release of CO2 over time. During the incubation, several parameters are analyzed (pH, salinity, temperature, oxygen, CO2). The study is maintained for 6 months. The biodegradation of the test material is considered to be achieved when it has reached a plateau.

Seawater	Sampling location	Coordinates GPS	Storage	Handling (sieve,…)	pH and salinity	Microorganism. mL^{-1}
Natural	Erquy	48.6344, -2.4779	Room temperature with ventilation, for 3 days	Addition of nutrients before incubation	8.2 35.2 %	4.8.10^5 Cellules.mL^{-1}

Table 2: Details of seawater sampled used for incubation

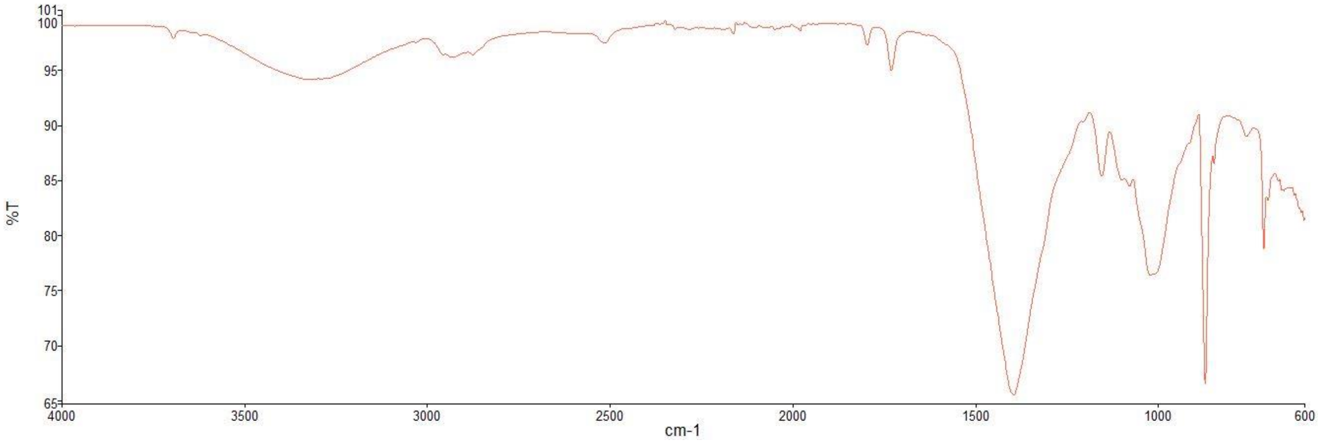
Internal Reference	Form	Dry matter (%)	TOC (%)	Observation
COA 20220218-01	Sequins	1%	38.47	
JRT 20220325-05	Sequins	1.2 %	39.90	
JRT 20220325-07	Sequins	1.1	38.01	

PHA sea live	Sequins	1.3	56.16	

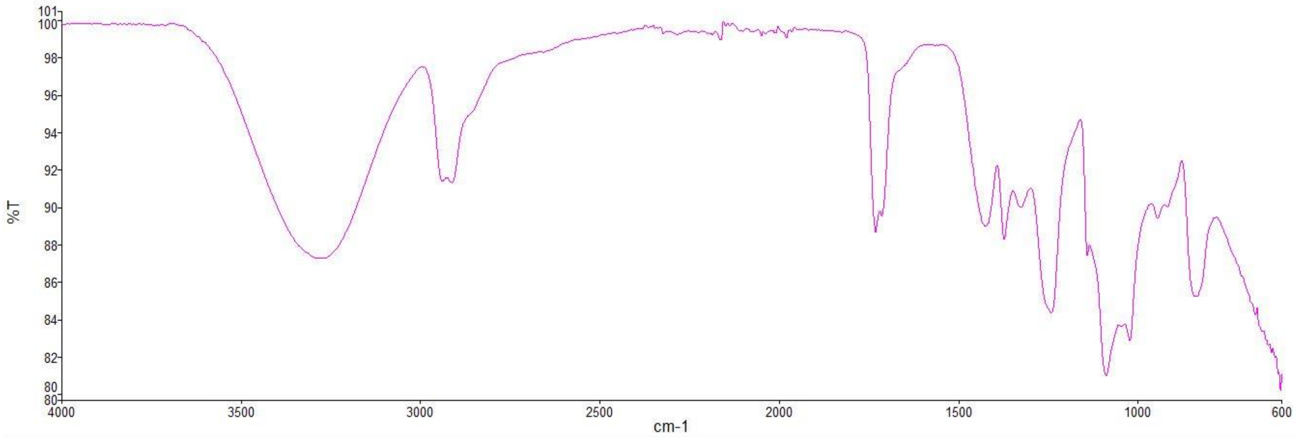
Table 3: Details of all the materials tested during the incubation

The infrared spectrum of the materials is presented below:

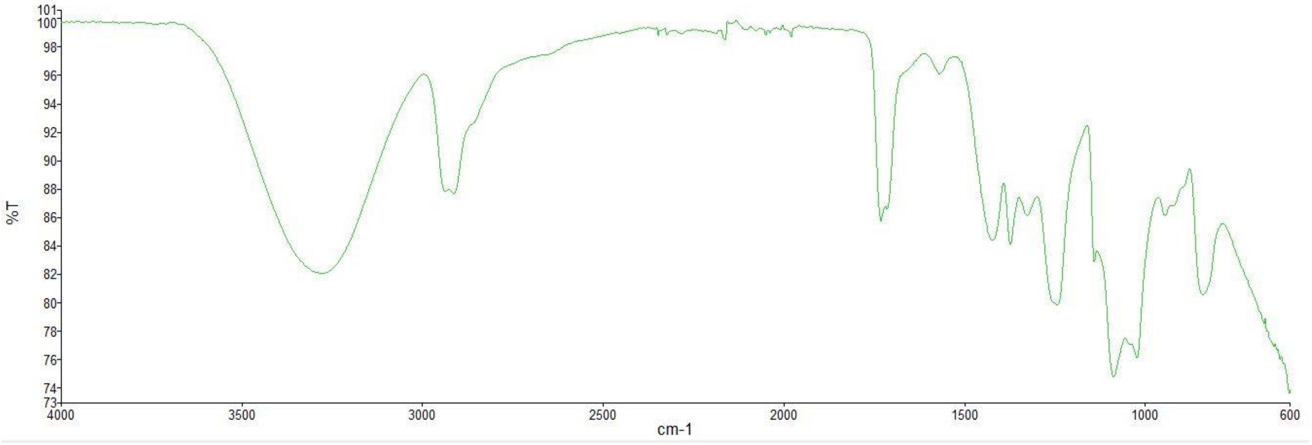
JRT 20220325-05	A4 sheet, cellulose paper with a thin layer of plastic	Ref. 02



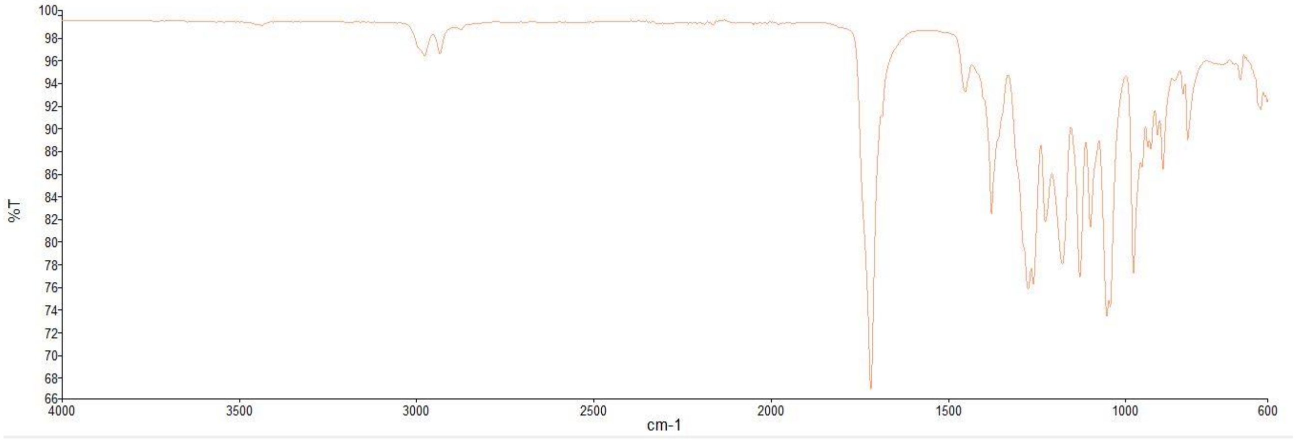
COA 20220218-01	A4 sheet, cellulose paper with a thin layer of plastic	Ref. 01



JRT 20220325-07	A4 sheet, cellulose paper with a thin layer of plastic	Ref. 03



PHA sea live	Form of powder	Ref. 04



Reference material: cellulose

The reference material used in the biodegradation test is microcrystalline cellulose (Sigma Aldrich, CAS 9004-34-6). It is a DFS-0, microcrystalline cellulose for thin-layer chromatography with a particle size between 0 and 40 µm.

2.2.2.1. Incubation

The different materials were added to seawater in accordance with the recommendations of the ASTM 6691 standard. The data is detailed in the table below.

Reference	total mass (g)	Total Carbon	Th CO2 (g)	Th CO2 (mg)
Cellulose 1	0,2318	0,4476	0,38043016	380,43016
Cellulose 2	0,2299	0,4476	0,37731188	377,31188
COA 20220218-01-1	0,2394	0,3847	0,33768966	337,68966
COA 20220218-01-2	0,2405	0,3847	0,339241283	339,2412833
JRT 20220325-05-1	0,2362	0,39905	0,345603903	345,6039033
JRT 20220325-05-2	0,2359	0,39905	0,345164948	345,1649483
JRT 20220325-07-1	0,2218	0,38015	0,309163323	309,1633233
JRT 20220325-07-2	0,2421	0,38015	0,337459155	337,459155

PHA sea live 1	0,2375	0,5616	0,48906	489,06
PHA sea live 2	0,2212	0,5616	0,45549504	455,49504

Table 4 : Calculation of Th CO2 for all the samples incubated for the study of the kinetics of biodegradation.

Incubation conditions:

Time (days)	**Temperature (°C)**	**Agitation**	**Salinity**	**Observation**
130	30	170 rpm	35.2	nothing to report

2.2.2.2. Kinetics of CO2 released over time

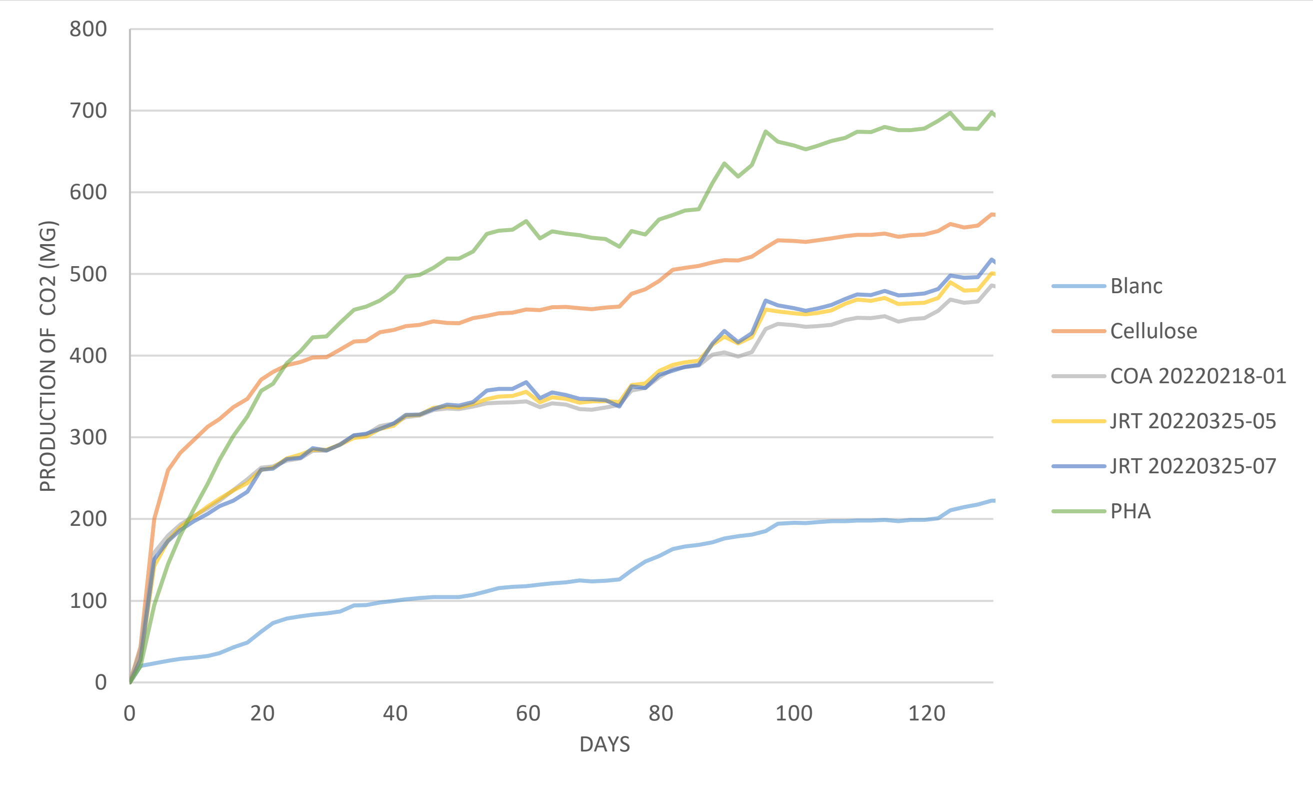


Figure 16: Graph representing CO2 production (in mg) as a function of time (in days)

CO2 production (in mg) over time was observed every four hours for 130 days (Figure 16). The white consistently produced 222.7 (+/- 10.5) mg of CO2 in 130 days. This control makes it possible to estimate the respiration of the microbial consortium alone, without adding carbon.

Cellulose, the positive control of the experiment, shows a significant production of CO2 over time. After 130 days, the cellulose produced an average of 572.9 (+/- 7.8) mg of CO2, i.e. a production twice as high as that of the white.

The COA 20220218-01 test has a CO2 production of 486 (+/- 14) mg CO2, the JRT 20220325-05 test has a CO2 production of 500.8 (+/- 26) mg CO2, the JRT 20220325-07 test has a CO2 production of 517.8 (+/- 19.8) mg of CO2 and finally the PHA has a CO2 production of 697.76 (+/- 29.2) mg of CO2 after 130 days of study.

2.2.2.3. Kinetics of biodegradation over time

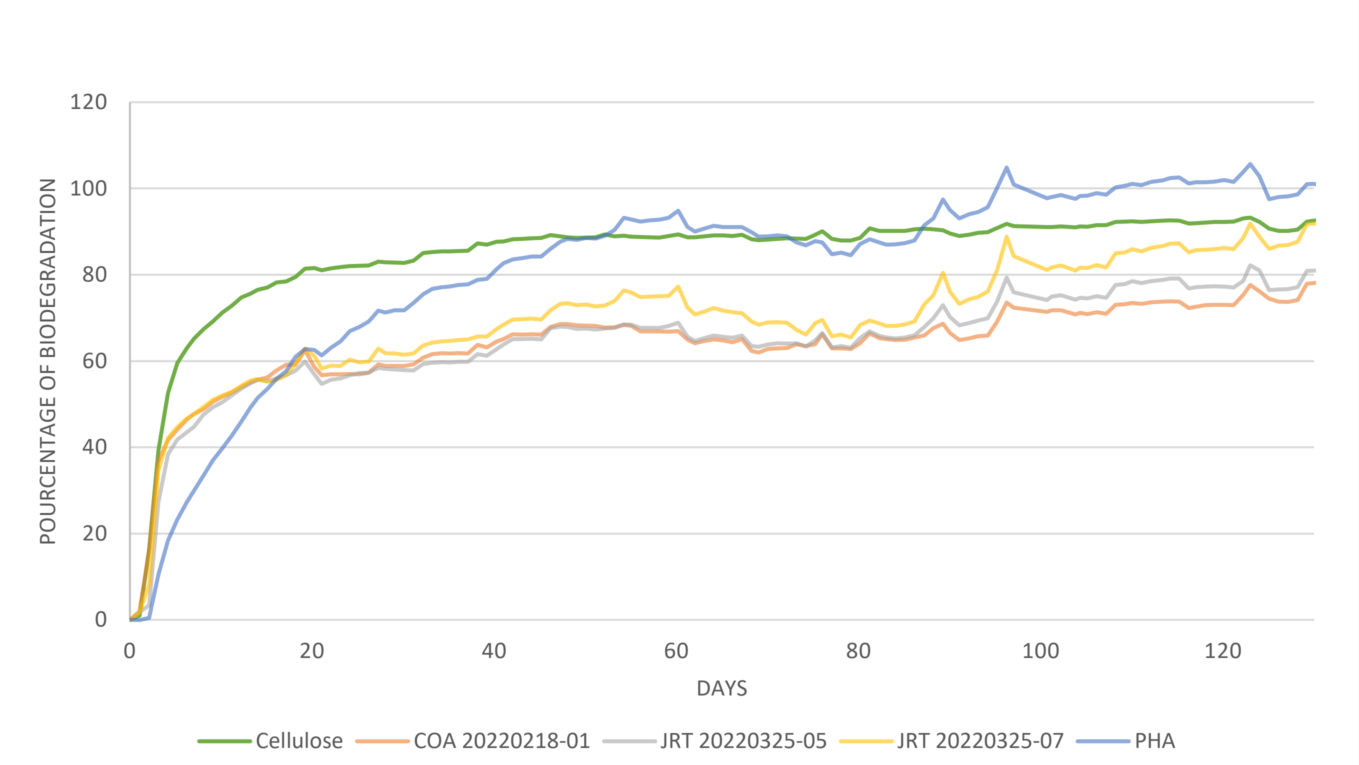


Figure 17 : Graph representing the percentage of biodegradation as a function of time (in days)

This graph represents the percentage of biodegradation of the various tests as a function of time in days (Figure 17).

Cellulose	**Mean**	**92.55**
	SD	2.5
COA 20220218-01	Mean	78.07
	SD	4.04
JRT 20220325-05	Mean	80.94
	SD	7.44
JRT 20220325-07	Mean	91.84
	SD	2.12
PHA	Mean	100
	SD	2.55

Table 5: Summary of biodegradation percentages obtained after 130 days

Cellulose shows a biodegradation percentage of 92.55% (+/- 2.5) in 130 days. This percentage of biodegradation makes it possible to prove that the microbial consortium is indeed active according to the ASTM 6691 standard.

COA sample 20220218-01 shows a biodegradation percentage of 78.07 % (+/- 4.04) after 130 days. This corresponds to 84.35% biodegradation relative to cellulose. **This sample cannot be considered biodegradable according to the ASTM 6691 standard.**

Sample JRT20220325-05 shows a biodegradation percentage of 80.94 % (+/- 7.44) after 130 days. This corresponds to 87.46 % biodegradation relative to cellulose. **This sample cannot be considered biodegradable according to the ASTM 6691 standard.**

Sample JRT20220325-07 shows a biodegradation percentage of 91.34% (+/- 2.12) after 130 days. This corresponds to 98.69 % biodegradation relative to cellulose. **This sample can be considered biodegradable according to the ASTM 6691 standard.**

The PHA sample shows a biodegradation percentage of 100% (+/- 2.55) after 130 days. This corresponds to 109% biodegradation relative to cellulose. **This sample can be considered biodegradable according to the ASTM 6691 standard.**

2.2.2.4. Conclusion

<u>Validity criteria:</u>

Average biodegradation rate of reference material > 70%? Yes

Difference between the percentages of biodegradation of the reference material in the triplicates at the end of the test < 20%? Yes

130 days	Percentage biodegrada tion	Standard deviation	Observation
Microcrystalline Cellulose	92.55	2.5	The cellulose was biodegraded within the time imposed in the ASTM 6691 standard. This means that the seawater studied has a sufficiently high microbial activity to validate the test.
COA 20220218-01	78.07	4.04	Sample considered non-biodegradable because does not reach 90% relative to cellulose biodegradation
JRT 20220325-05	80.94	7.44	Sample considered non-biodegradable because does not reach 90% relative to biodegradation cellulose
JRT 20220325-07	91.84	2.12	Sample that can be considered as biodegradable because it reaches 90% biodegradation relative to cellulose required by standard ASTM 6691
PHA sea live	100	2.55	Sample that can be considered biodegradable because it reaches the 90% absolute biodegradation required by the ASTM 6691 standard

2.2.3. Sonae

This study aims to determine the degree of disintegration of plastic materials in a laboratory-scale industrial aerobic composting process. The suitability for domestic composting will be effective when a material meets several criteria according to the EN13432 standard:

- Its biodegradation was assessed according to EN ISO 14855-1 (March 2013)
- Its disintegration evaluate according to EN ISO 16 929 (March 2021) or EN ISO 20 200 (Feb 2016)
- Its non-toxicity according to the OECD 208 test standard (July 19, 2006)

The test material is mixed with fresh biological waste and introduced into a defined composting environment at 58°C for the first 90 days and 25°C for the next 90 days. The composting process is monitored over time (temperature, pH, and moisture content) to allow the activation of the naturally present microbial population. The study is maintained over a period of between 45 days and 90 days, the time required to obtain stable compost. This duration can be extended to 180 days if signs of disintegration are still ongoing. At the end of the test, the maturity of the compost is determined, and the plastic residues are examined on 2 mm sieves, which makes it possible to assess the degree of disintegration.

Sonae reference		Date of reception
Reference	**Type**	
TC paper	Unmodified control kraft paper	10/13/2021
T1 paper 1 layer	Paper series 1: eco Kraft paper (73g/m^2) coated with a layer of 3 to 5µm of Ethylcellulose 5.4 g/m^2	10/13/2021
T2 paper 2 layers	Paper series 2: Paper Eco Kraft with an ethylcellulose layer + Cellulose + Ethylcellulose	10/13/2021

Table 5 : Identification of plastics to be studied

2.2.3.1. Experimental condition

<u>Insulated composters</u>
In the laboratory, the reactors used are made of polypropylene (PP), having the following dimensions: 32 cm x 22 cm x 11 cm (L, W, H). The box is associated with a lid. The box has a volume of 5.2 L. The incubators are identified as ISO 20200 incubators.

<u>Bio-waste:</u>
The bio-waste used is:

- Sawdust (untreated) (40%)
- Rabbit food (30%)
- Mature compost (10%)
- Corn starch (10%)
- Sucrose (5%)
- Corn oil (4%)
- Urea (1%)
- Distilled water (QSP 50% humidity)

This mixture of bio-waste was analyzed:

- Humidity: 51.2%
- pH: 6.4
- volatile solids: 0.945 g/g

Material	Sample name	Reception	Description	Form / Appearance	Thickness (mm)	Dimensions for analysis (mm)	Photography
TC paper	Unmodified control kraft paper	10/13/2021	Papier eco kraft (112µm; 73g/m²)	flat film	112 µm	4,5*4,5*0,112	
T1 paper 1 layer	Paper series 1: eco Kraft paper (73g/m²) coated with a layer of 3 to 5µm of Ethylcellulose 5.4 g/m²	10/13/2021	Papier eco kraft (112µm; 73g/m²)+ethylcellulose 3,4g/m²+Ehtylcellulose (3 à 5 µm) representing 4 to 7% of plastic	flat film	117 µm	4,5*4,5*0,117	
T2 paper 2 layer	Paper series 2: Paper Eco Kraft with an ethylcellulose layer + Cellulose + Ethylcellulose	10/13/2021	Papier eco kraft (112µm; 73g/m²)+with an ethylcellulose layer +Cellulose+ Ethylcellulose, Ethylcellulose represents 8% of plastic	flat film	132 µm	4,5*4,5*0,132	

Table 6 : Characteristics of the materials used in this study

The experiment began on October 11, 2021, and ended on April 09, 2022. The duration of this experiment was 180 days, the maximum time allowed by the ISO 20200 standard. For this study, 25 slides were used per plastic tested and put in bio-waste in ISO 20200 incubators. The shape of the plastics studied, and the volatile solids are given in Table 4 of this report. Incubation was performed at 58°C for the first 90 days and 25°C for the last 90 days.

The experimental follow-up is carried out according to the indications of the standard.

2.2.3.2. Results

Characteristic	***Time***	***Theoretical***	***Observation Real***
Odor	2 to 3rd days	ACID	YES
	5 to 10th days	AMMONIA	YES
	20th day	EARTH	YES
Visual aspect	1st week	Mycelial Development	YES
	1 to 9th days	Yellow color	YES
	From the 10th day	Brown color	YES
Chemical analysis	Synthetic waste (beginning) Compost (end of incubation) after sieving (2 mm)	Total carbon/total nitrogen (C/N) and pH	OK

Table 7 : Main characteristics of the experimental follow-up of this study

Monitoring the disintegration of plastic by photography

In this study we have analyzed three types of plastics, the photographs below show the evolution of these three types of plastics

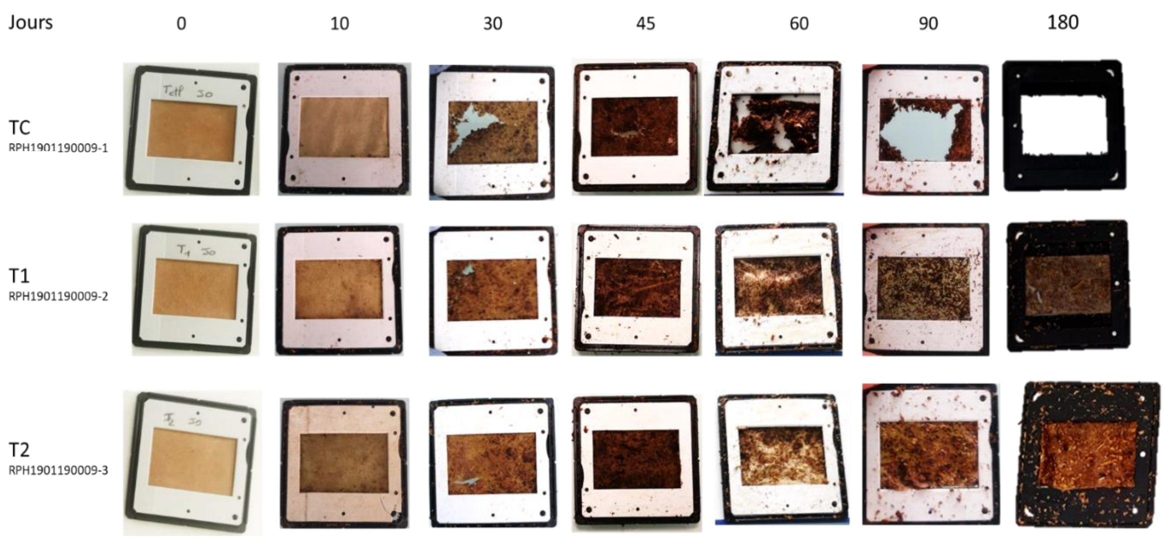


Figure 18 : Evolution of TC, T1 and T2 samples during incubation

During the entire composting process in our incubators, **only the control paper reference containing no layer of PVOH completely disintegrated with the naked eye.** The slides are virgin plastic and are not found in the compost.

Plastic T1 and T2 have not disintegrated. Traces of disintegration are visible, but this does not allow these two samples to be characterized as disintegrable. Some plastic parts have come off the slide during the many interviews of the experiment. **However, these pieces are intact in the compost and cannot be considered disintegrated.**

Monitoring of the composting process during the ISO 20200 incubation

The monitoring of the composting process is presented in the figure below (Figure 19):

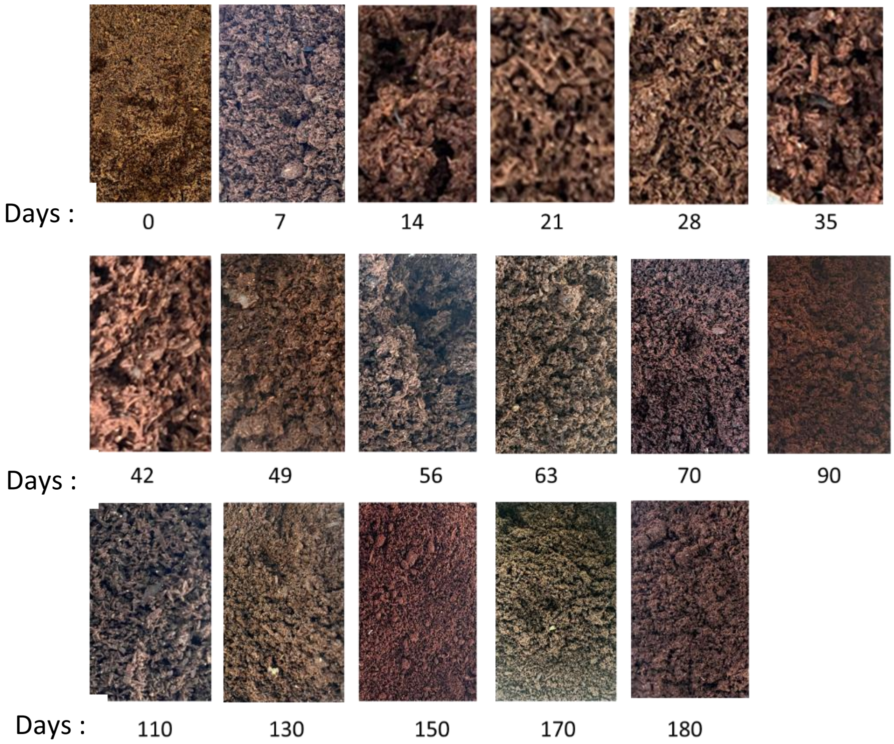


Figure 19: Evolution of bio-waste in compost according to the ISO 20200 standard over the 180 days of study

End of incubation date: 14/11/2022 (181 study days)

Sample	**Nitrogen percentage (%)**	**Carbon percentage (%)**	**C/N ratio**	**Volatile solids in (g/g)**
Compost + Tc material	1,85	44,78	24,21	0,80
Compost + Tc material	2,14	45,41	21,22	0,77
Days : t + Tc material	2,13	44,69	20,98	0,73
Compost + T1 material	2,16	45,7	21,16	0,74
Compost + T1 material	2,14	43,22	20,20	0,76
Compost + T1 material	2,21	45,96	20,80	0,72
Compost + T2 material	2,03	45,76	22,54	0,75
Compost + T2 material	2,14	44,1	20,61	0,75
Compost + T2 material	2,14	44,96	21,01	0,77

Table 8 : Physico-chemical characteristics of the compost at the end of the experiment, after 180 days of incubation

The analyses show that after 180 days the compost containing the test materials has a C/N ratio between 20:1 and 24:1.

Days :

Bioreactor	Samples	Bioreactor mass + Wet compost	Wet compost mass	Moisture percentage (%)	Drying time (h)	Post-experiment dried mass with compost + Plastic	Compost moisture (%)	pH	Disintegra-ted surface	Standard deviation
1	Compost + Tc material	1048,4	748,4	55,5	48	747,5	0,5	6,8	100	0
2	Compost + Tc material	1112,5	812,3	60,1	48	523,69	1,1	6,6	100	0
3	Compost + Tc material	1106,2	806,1	57,3	48	639,2	0,9	7,1	100	0
4	Compost + T1 material	1110,1	810	58,5	48	648,6	0,89	7,9	0	0
5	Compost + T1 material	1113,9	813,7	59,9	48	524,3	1,23	6,4	0	0
6	Compost + T1 material	1109,2	809,1	56,2	48	652	1,12	6,3	0	0
7	Compost + T2 material	1126,5	826,5	58,9	48	545,2	0,79	7,3	0	0
8	Compost + T2 material	1116,2	816	59,2	48	537	0,97	7,5	0	0
9	Compost + T2 material	1107	806,9	61	48	509,7	1,25	7,2	0	0

Table 9: Study of plastic disintegration during the ISO 20200 study (180 days).

The results show that after 180 days, only the Tc plastic showed complete disintegration. The other plastics T1 and T2, have their surfaces almost intact within 180 days. The loose pieces of the slides are weakened and brittle, but still visible in the compost. They were therefore not counted as disintegrated.

Sample	Initial mass in the incubator (g)	Dry matter of initial bio-waste (%)	Initial compost volatile solids (%)	Final mass of compost (g)	Compost dry matter	Compost Final Volatile Solids (%)	R
Tc-1	1001	48,8	94,57536723	748,4	44,5	79,94890511	42,36658227
Tc-2	1007,8	48,8	94,57536723	812,3	39,9	76,62854301	46,60412537
Tc-3	1001,9	48,8	94,57536723	806,1	42,7	72,9401611	45,70481972
T1-1	1003,9	48,8	94,57536723	810	41,5	73,81575259	46,44576068
T1-2	1005,8	48,8	94,57536723	813,7	40,1	75,68639053	46,79934328
T1-3	1003,9	48,8	94,57536723	809,1	43,8	71,82863715	45,06038325
T2-1	1006	48,8	94,57536723	826,5	41,1	74,53978159	45,46479417
T2-2	997,8	48,8	94,57536723	816	40,8	75,20905923	45,62752276
T2-3	994,9	48,8	94,57536723	806,9	39	77,03573004	47,20421691

Table 10: Evolution of volatile solids during incubation

According to the ISO 20200 standard, for the experiment to be valid, the R must be greater than or equal to 30%, this R depends on the evolution of the compost over time, its dry matter, and volatile solids. The results of this experiment are shown in Table 10.

All the composts studied to make it possible to validate this criterion of the standard.

2.2.3.3. Conclusion

<u>Validity criteria of the EN ISO 20200 standard</u>

- The degree of decay of the three replicates must not have a standard deviation greater than 20%
- The decrease in volatile solids between the beginning and the end of the experiment must be greater than 30%
- The disintegration of the material must be greater than 90%

<u>Disintegration criteria according to EN ISO 20200</u>

In our laboratory conditions, respecting the EN ISO 20200 standard (2015 version), the TC plastic (RPH1901190009-1) described in paragraph 1 of this report, can be considered as disintegrable because it has been observed with a disintegration percentage of 100 % and a decrease in volatile solids on the compost of 44.89% on average.

In our laboratory conditions, respecting the EN ISO 20200 standard (2015 version), the T1 (RPH1901190009-2) and T2 (RPH1901190009-3) plastics described in paragraph 1 of this report cannot be considered disintegrable. The composting process cannot be called into question because the physicochemical characteristics of the compost, as well as the reduction in R (46.1 (+/-0.69)% and 46.1(+/-0.73)% for T1 and T2 plastics respectively), are met according to the criteria of the standard.

2.3. Accreditation by TÜV Austria

2.3.1. TÜV Austria company

TÜV Austria is a company of more than 2000 people which is established in more than 20 countries. They offer solutions for industrial service, inspection, monitoring, certification, IT security, insurance services and training. The international orientation of the TÜV AUSTRIA group and the multitude of national and international accreditations make TÜV AUSTRIA a competent, safe and reliable partner. TÜV AUSTRIA's OK environment product verification marks are the only ones of their kind to offer a customized certification label for each biodegradation environment.

2.3.2. Audit TÜV Austria at IPC

IPC worked for 2 years to have TÜV Austria accreditation.

For this, industrial, domestic, and marine compost tests have been set up according to TÜV Austria specifications.

All the experiments included in the TÜV Austria Ok home compost and OK industrial compost accreditation have been set up:

- Polymer quality
- Biodegradation according to ISO 14855-1
- Disintegration at laboratory scale (ISO 20200) and pilot scale (ISO 16929)
- Ecotoxicity according to OECD 208

The first pre-audit took place in December 2021. This pre-audit highlighted the following non-conformities:

- The entire fleet of machines used for biodegradation tests had to be calibrated by a certified organization
- The temperature had to be respected for the ISO 629 disintegration test in industrial compost.
- The biodegradation of cellulose in the marine environment had to wait for at least 70% biodegradation
- The ecotoxicity test had to allow the germination of at least 90% of the plants in the reference medium.
- Advice was given for writing the operating mode and the test report
- the archiving of test plastics was to last 3 years

These non-conformities were all dealt with the following year.

To respond to these non-conformities, changes have taken place in our operating methods:

- A service provider was found and allowed the calibration of all the devices. This calibration must take place every year.

- To allow compliance with the temperatures in the ISO 16929 standard, heating blankets have been purchased which surround the bio-waste tanks. These blankets have the role of maintaining the temperature which makes it possible to remain within the temperatures constrained by the standard.
- To achieve 70% cellulose biodegradation (positive control), the seawater used as the incubation medium was changed. This made it possible to select another type of bacterial consortium and to achieve the 70% biodegradation required in the ASTM 6691 standard (biodegradation in the marine environment).
- The origin of wheat and mustard seeds has been changed as well as the soil used as reference soil. This allowed to have 90% germination of the seeds.
- The advice has been followed to increase understanding of the operating mode and to follow the recommendations of the TÜV Austria organization.
- A larger storage area has been set up to allow the storage of all the samples.

The following year (2022) allowed IPC to accumulate experience and positive results according to the specifications of TÜV Austria. The audit took place on December 7 and 8, 2022. For two days, the auditor observed the administrative and technical documents and the implementation of standards within the IPC laboratory.

This audit was a success for IPC, leading to TÜV Austria certification for industrial compost and domestic compost.

ZERTIFIKAT | CERTIFICATE | CERTIFICAT | CERTIFICADO | СЕРТИФИКАТ | شهادة | 证书 | 인증서

TÜV AUSTRIA

REGISTRATION

TA8092207170

FOR TESTING ACCORDING TO

OK compost TÜV AUSTRIA INDUSTRIAL

Issued by TÜV AUSTRIA Belgium NV to

CT-IPC
Biopôle Clermont-Limagne
5 rue Emile Duclaux
63360 Saint-Beauzire
FRANCE

for the following tests

Biodegradation

Disintegration

Ecotoxicity

Heavy Metals and Fluorine

The tests comply with the requirements described in:

Certification Scheme
Compostability of products (OK 1 Edition F)
Including EN 13432 (09-2000)

Validity of the certificate from 22 December 2022 till 22 December 2027

Brussels, 22 December 2022

For the Certification Committee
Ph. DEWOLFS
President of the Committee

FM-LTC-TABE-CERT-BIO-OKI-004_certificate_lab_EN
Rev 1906

028065-21-6

Figure 20: TÜV Austria Accreditation for IPC Industrial Compost

ZERTIFIKAT | CERTIFICATE | CERTIFICAT | CERTIFICADO | СЕРТИФИКАТ | شهادة | 证书 | 인증서

TÜV AUSTRIA

REGISTRATION

TA8092207172

FOR TESTING ACCORDING TO

Issued by TÜV AUSTRIA Belgium NV to

CT-IPC
Biopôle Clermont-Limagne
5 rue Emile Duclaux
63360 Saint-Beauzire
FRANCE

for the following tests

Biodegradation

Disintegration

Ecotoxicity

Heavy Metals and Fluorine

The tests comply with the requirements described in:

Certification Scheme
Home compostability of products (OK 2 Edition D)

Validity of the certificate from 22 December 2022 till 22 December 2027

Brussels, 22 December 2022

For the Certification Committee
Ph. DEWOLFS
President of the Committee

FM-LTC-TABE-CERT-BIO-OKH-004_certificate_lab_EN
Rev 1906

028063-21-6

Figure 21: TÜV Austria Accreditation for IPC home Compost

TÜV marine certification was also assessed. This certification will take place in a second phase after an additional verification experience.

As TÜV Austria is also authorized to carry out an audit for the "Seedling" accreditation, IPC has also been assessed on this point. This accreditation is very similar in terms of requirements to the industrial compost accreditation of TÜV Austria


ZERTIFIKAT | CERTIFICATE | CERTIFICAT | CERTIFICADO | СЕРТИФИКАТ | شهادة | 证书 | 인증서

TÜV AUSTRIA

REGISTRATION

TA8092207171

FOR TESTING ACCORDING TO

Issued by TÜV AUSTRIA Belgium NV to

CT-IPC
Biopôle Clermont-Limagne
5 rue Emile Duclaux
63360 Saint-Beauzire
FRANCE

for the following tests

Biodegradation
Disintegration
Ecotoxicity
Heavy Metals and Fluorine

The tests comply with the requirements described in:
Certification Scheme
Products made of compostable materials (Edition 2020-01)

Validity of the certificate from 22 December 2022 till 22 December 2027

Brussels, 22 December 2022

For the Certification Committee
Ph. DEWOLFS
President of the Committee

FM-LTC-TABE-CERT-BIO-SEE-004_certificate_lab_EN
Rev 1906

028064-21-6

Figure 22: Seedling accreditation issued by TÜV Austria for IPC

3. Conclusions

IPC progressed for two years in the framework of the FlexFunction2Sustain project to upgrade and obtain the TÜV Austria accreditation for the Biodegradix pilot line. In addition, several materials were purchased, such as screens, chemicals, and light laboratory equipment for ecotoxicity testing. Thanks to the project, the upgrades could have been tested and validated for the TÜV Audit.

Indeed, this pilot line is able for evaluating biodegradation in domestic compost, in industrial compost according to the specifications of TÜV Austria. All of the protocol steps (quality, biodegradation, disintegration, and ecotoxicity) are studied at IPC. A study was also carried out to set up tests in the marine environment.

Currently, IPC is able to carry out a biodegradation study project in the marine environment, and certification with the TÜV Austria organization is in progress (OK marine environment).

4. Degree of progress

Deliverable 3.4 is fulfilled by 100 %

5. Dissemination level

Public