

FINAL REPORT

**Biodegradation study of pure P3HB and P3HB and PLA blends in sea
water**

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SUMMARY

1 Study title

Biodegradation study of polylactic acid (PLA) and polyhydroxybutyric acid (P3HB) conducted on P3HB powder and PLA-P3HB based blend.

2 Biodegradation test

2.1. Test condition

Concentration ThOD* of tested materials	100 mg/L
Test suspension volume	300 mL
Test temperature	22 °C
Test period	169 days

*Theoretical oxygen demand

2.2. Measurements and analyses

Measurement of the biochemical oxygen demand with closed system oxygen consumption measuring apparatus.

2.3. Test results

Material	Degradation [%]
P3HB powder (pure)	30.0
Foil of blend containing more than 50% of P3HB	17.6
Foil of blend containing more than 50% of PLA	9.9
Positive control – microcrystalline cellulose	46.4

FINAL REPORT

3 Study title

Biodegradation study of polylactic acid (PLA) and poly-3-hydroxybutyric acid (P3HB) conducted on P3HB powder and PLA-P3HB based blend.

4 Sponsor

NAFIGATE Corporation, a.s.

5 Testing facility

Name	Biodegradation of Bioplastics Laboratory, Institute of Chemistry and Technology of Environmental Protection, Brno University of Technology
Address	Purkyňova 464/118, 612 00 Brno

6 Purpose of study

This study was conducted to evaluate biodegradability of the P3HB powder and foils consisting of blends containing more than 50% of either P3HB or PLA.

7 Test method

This study was conducted in accordance with the ISO 16221:2001 „Water quality — Guidance for determination of biodegradability in the marine environment“.

8 Test period

Start of the test	July 4th 2018
End of the test	December 19th 2018
End of study	September 20th 2019

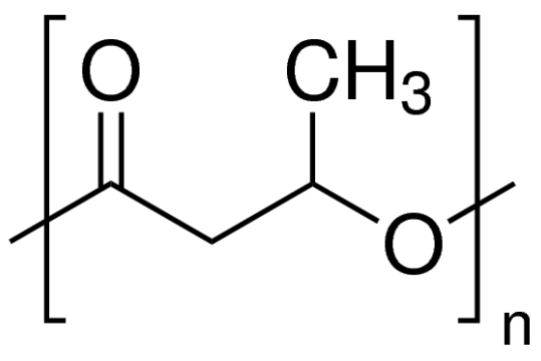
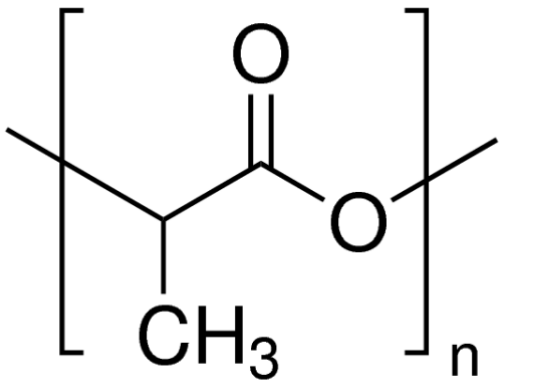
9 Test substances

P3HB is natural substance. PLA is synthetic substance made from natural resources. Both are considered biopolymers in this study. Test substances described herein is identified by following name, structures, etc.

9.1. Name

Poly-3-hydroxybutyric acid, polylactic acid

9.2. Structure

P3HB	PLA
	

9.3. Molecular formula:

P3HB	$[C_4H_6O_2]_n$
PLA	$[C_3H_4O_2]_n$

9.4. Composition of tested materials:

P3HB powder	100% P3HB
foil of blend containing more than 50% of P3HB	P3HB, PLA and other additives
foil of blend containing more than 50% of PLA	P3HB, PLA and other additives

9.5. Adjustment of the foils

Foils containing more than 50% of P3HB and PLA were cut into squares with area of approximately 0.5 cm².

9.6. Storage conditions

All tested materials were stored at cool and dark place at a temperature of 22 °C. P3HB powder was stored in a dark glass bottle. Both foils were stored in closed plastic bags.

10 Conduction of the biodegradation study

10.1. Sea water collection

Sea water was collected near Slovenian town Portorož (GPS 45°30'46.6"N 13°35'06.3"E) at various places from a depth of 75 cm on the 25th of June, 2018 at 10:30 a.m. Water was stored in plastic canisters during the transport to the laboratory, where the water was stored in a glass aquarium and aerated with air pump.

10.2. Sea water parameters

Parameter	
Conductivity	79,3 mS
pH	7,83
Salinity	3,83 %
Phosphorus (as phosphates)	1,3 mg/l
Nitrogen (as ammonia)	0,34 mg/l

10.3. Preparation of test medium

Test medium was prepared in a volumetric flask in accordance with ISO 14851:1999. 300 mL of the test medium was used in each test flask.

10.4. Reference material

Microcrystalline cellulose (ZVC Dr. Hoffmann, Cítov, ČR).

10.5. Preparation of suspensions

TEST SUSPENSION:

300 mL of the test medium was transferred to a reaction. Test materials were added to make ThOD concentration of 100 mg/L. The bottles were hermetically sealed. Each experiment was conducted twice.

BLANK EXPERIMENT:

The procedure was the same as in the test experiment except for the test material was not added.

POSITIVE CONTROL:

The procedure was the same as in the test experiment, but instead of test material microcrystalline cellulose was added. In accordance with ISO 14851:1999, this test was conducted only once.

10.6. Measuring equipment and procedure

Equipment:

Oxygen electrode GMH 3651 from GHM-Greisinger

Laboratory shaker GFL 3005

Procedure

Test solutions were saturated with oxygen by using compressed air. Each bottle was shaken by using laboratory shaker to prevent activated sludge sedimentation. Next day, a decrease in oxygen content in each bottle was measured. Then the suspensions were saturated with oxygen again. Percentage of material biodegradation was calculated from measured oxygen demand, i.e. from a decrease of oxygen used by microorganisms.

10.7. Test condition

Temperature	22 °C
Test period	169 days
Speed of lab shaker	250 rpm
Speed of lab mixer during oxygen measurement	200 rpm

10.8. Calculation of biodegradability

$$\text{degradability(\%)} = \frac{BOD - B}{ThOD} \cdot 100$$

where

BOD is biochemical demand of the test culture

B is biochemical oxygen demand of inoculum blank

ThOD is theoretical oxygen demand required for complete oxidation of the test substance

11 Results

11.1. Appearance of the test suspensions at the start of test

Test suspensions	Appearance
P3HB powder (pure)	Transparent suspension with visible P3HB particles
Foil of blend containing more than 50% of P3HB	Transparent suspensions with foil squares
Foil of blend containing more than 50% of PLA	Transparent suspensions with foil squares
Inoculum blank	Transparent suspension
Positive control	Transparent suspension with visible cellulose particles

11.2. Appearance of test suspensions at the end of the test

Test suspensions	Description of the appearance
P3HB powder (pure)	Transparent suspension
Foil of blend containing more than 50% of P3HB	Transparent suspension with visible foil particles
Foil of blend containing more than 50% of PLA	Transparent suspension with visible foil particles
Inoculum blank	Transparent suspension
Positive control	Transparent suspension

11.3. Results of test

Material	Degradation [%]
P3HB powder (pure)	30.0
Foil of blend containing more than 50 % of P3HB	17.6
Foil of blend containing more than 50 % of PLA	9.9
Positive control – microcrystalline cellulose	46.4

12 Conclusion:

The highest percentage of biodegradation reached positive control (46.4%), the PLA majority foil (9.9 %) reached the lowest percentage of biodegradation. The rate of biodegradation of all samples was significantly slower due to low microbial content.

13 Biodegradation graph

