BRNO UNIVERSITY OF TECHNOLOGY
FACULTY OF CHEMISTRY

FINAL REPORT

Biodegradation study of pure P3HB and P3HB and PLA blends

CONTENT

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
Activated sludge concentration	500 mg/L (as suspended solid)
Test suspension volume	300 mL
Test temperature	22 °C
Test period	137 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 [%]**	**Normalized degradation [%]**
P3HB powder (pure)	88.4	96.3
Foil of blend containing more than 50 % of P3HB	88.9	96.8
Foil of blend containing more than 50 % of PLA	33.7	33.7
Positive control – microcrystalline cellulose	91.8	100

**reflects accumulative measurement error of repeated measurements. The reference material was 100 % degraded, as followed from the degradation curve (reached plateau). Based on this observation, the tested samples were recalculated.*

FINAL REPORT

1. Study title

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

2. Sponsor

NAFIGATE Corporation, a.s.

3. 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

4. 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.

5. Test method

This study was conducted in accordance with the ISO 14851:1999 „Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium-method by measuring the oxygen demand in a closed respirometer“.

6. Test period

Samples of activated sludge taken at WWTP Modřice	April 6 2018
Start of the test	April 9 2018
End of the test	August 24 2018
End of study	September 3 2018

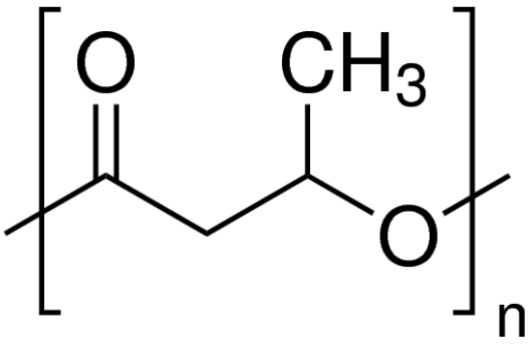
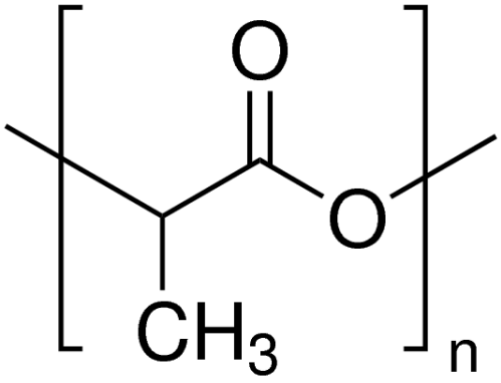
7. Test substances

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

7.1. Name

Poly-3-hydroxybutyric acid, polylactic acid

7.2. Structure

P3HB	PLA
	

7.3. Molecular formula

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

7.4. 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

7.5. Adjustment of the foils

Foils containing more than 50% of P3HB and PLA were cut into squares with area approximately 0.5 cm^2.

7.6. Storage conditions

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

8. Conduction of the biodegradation study

8.1. Activated sludge

Activated sludge was taken at wastewater treatment plant Modřice (population equivalent 513 000) in PTFE bottles and stored in a fridge at 4±2°C.

8.2. Determination of concentration of suspended particles of activated sludge.

Concentration of activated sludge suspended particles was determined in accordance with ISO 11923:1997 „Water quality - Determination of suspended solids by filtration through glass fibre filters“. Briefly, in the first step, filters Filpap Z7 (55 mm in diameter) were washed by using distilled water and dried in a lab dryer at 105 °C for 90 minutes. Then, two of them were used for filtration of activated sludge and one was used to filter distilled water as a control. Filters were dried at the same condition as before and concentration of suspended particles of was calculated from mass gain. As a result, 6106 mg L^{-1} of suspended particles per L was determined

8.3. Preparation of test medium

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

8.4. Reference material

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

8.5. Preparation of suspensions

TEST SUSPENSION:

300 mL of test medium was transferred to a reaction bottle and activated sludge was added to reach concentration of suspended particles of 500 mg/L. Test materials were added to make ThOD concentration of 100 mg/L. The bottles were hermetically sealed. Each experiment was conducted twice.

INOCULUM BLANK:

Procedure was the same as the test suspension except for the test material was not added.

POSITIVE CONTROL:

Procedure was the same as for the test suspension, but instead of test material was added microcrystalline cellulose. In accordance with ISO 14851:1999, this test was conducted only once.

8.6. Measuring equipment and procedure

Equipment:

Oxygen electrode GMH 3651 from GHM-Greisinger

Laboratory shaker GFL 3005

Procedure

Test suspensions 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.

8.7. Test condition

Temperature	22 °C
Test period	137 days
Speed of lab shaker	250 rpm
Speed of lab mixer during oxygen measurement	200 rpm

8.8. 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

8.9. Validity of test

Validity of the test was confirmed by the degradability of microcrystalline cellulose after 137 days 91,8 %

9. Results

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

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

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

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

9.3. Results of test

Material	Degradation [%]	Normalized degradation [%]
P3HB powder (pure)	88.4	96.3
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10. Conclusion

Test confirmed biodegradation of foil with P3HB majority and P3HB powder. Rate of biodegradation of these materials were almost the same even though the foil with P3HB majority contained small amount of PLA. Biodegradation of foil with PLA majority was not confirmed, this material was only disintegrated under experimental conditions.

11. Biodegradation graph