

FINAL REPORT

Biodegradation study of P3HB in soil

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SUMMARY

1 Study title

Biodegradation study of polyhydroxybutyric acid (P3HB) conducted on P3HB powder.

2 Biodegradation test

Test condition

Concentrations of tested materials	1%
Amount of soil	5 g
Test temperature	20,00±0,01 °C
Test period	176 days

Measurements and analyses

Measurement of the released carbon dioxide in closed respirometer Respicond VI.

Test results

Soil	Degradation [%]
Soil 1	112.6
Soil 2	116.5
Soil 3	94.7

FINAL REPORT

3 Study title

Biodegradation study of poly-3-hydroxybutyric acid (P3HB) conducted on P3HB powder.

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.

7 Test method

This study was conducted in accordance with the ISO 17556:2012 „Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of evolved carbon dioxide“.

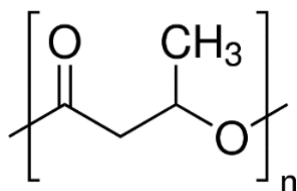
8 Test substances

P3HB is natural substance considered biopolymer in this study. Test substance described herein is identified by following name, structures, etc.

Name

Poly-3-hydroxybutyric acid, polylactic acid

Structure



Molecular formula:

P3HB	$[C_4H_6O_2]_n$
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Composition of tested materials:

P3HB powder	100% P3HB
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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.

9 Soils used for tests

Soils were collected in the Hovorany and Mikulčice villages.

	Soil 1	Soil 2	Soil 3
Soil type	chernozem	colluvium	fluvisol
pH	7,49	7,29	6,13
Calcium content	2,36%	5,63%	-
Silicon content	12,47%	8,54%	-
Humus content	1,75%	1,55%	0,94%
Humus quality	0,95	1,08	-

10 Conduction of the biodegradation study

Preparation of test sample

All three soils were air dried. Then 5 grams of each soil were weighted into glass beakers and distilled water was added to reach 76% of field capacity 1.8. P3HB was added directly in the beaker and mixed well. Four beakers with each soil test sample were prepared.

Preparation of blank sample

The procedure was the same as the test sample except for the test material was not added. There were prepared 3 blank experiments for each of the soils.

11 Measuring equipment and procedure

Equipment:

Respicond VI

Procedure

Beakers with test samples and blank samples were placed in the respirometer Respicond VI. Carbon dioxide that evolved during biodegradation reacted with potassium hydroxide solution in a smaller cup under the lid of testing vessel to produce potassium carbonate. This led to a change in conductivity of potassium hydroxide solution, which was automatically measured by conductometer with Pt electrodes. Potassium hydroxide was replaced when it reacted with 80 mg carbon dioxide.

Test condition

Temperature	20,00±0,01 °C
Test period	176 days
Concentration of potassium hydroxide	0,6 mol·dm ⁻³
Volume of hydroxide	10 ml

Calculation of biodegradability

$$ThCO_2 = \frac{M_r(CO_2)}{A_r(C)} \cdot m \cdot w_c$$

Where

ThCO₂ is theoretical amount of carbon dioxide evolved by test material

M_r(CO₂) is relative molecular mass of carbon dioxide

A_r(C) is relative atomic mass of carbon

m is mass of the test material, in milligrams, introduced in the system

w_c is carbon content of the test material, determined from the chemical formula, expressed as a mass fraction

$$\text{degradability}(\%) = \frac{\sum m_t - \sum m_B}{ThCO_2} \cdot 100$$

where

Σm_t is amount of carbon dioxide, in milligrams, evolved in the test vessel

Σm_B is the amount of carbon dioxide, in milligrams, evolved in the blank test vessel

12 Results

Results of test

Soil	Degradation [%]
Soil 1	112.6
Soil 2	116.5
Soil 3	94.7

13 Conclusion:

Test confirmed biodegradation of P3HB powder in all three soils. The results above 100% were caused by experimental error.

14 Biodegradation graph

