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**Ugotavljanje dokončne biorazgradnje plastičnih materialov v vodnem sistemu pri anoksičnih (denitrifikacijskih) pogojih - Metoda z meritvijo zviševanja tlaka**

Determination of the ultimate biodegradation of plastics materials in an aqueous system under anoxic (denitrifying) conditions - Method by measurement of pressure increase

Bestimmung der vollständigen Bioabbaubarkeit von Kunststoff-Materialien in wässriger Phase unter anoxischen (denitrifizierenden) Bedingungen - Verfahren mittels Messung der Druckzunahme

Détermination de la biodégradation ultime des matériaux plastiques dans un système aqueux dans des conditions anoxiques (dénitrifiantes) - Méthode par mesure de l'augmentation de pression

**Ta slovenski standard je istoveten z: prEN 17417**

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Kunststoffmaterialien in einem wässrigen System  
unter anoxischen (denitrifizierenden) Bedingungen -  
Verfahren durch Messung des Druckanstiegs

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<b>Contents</b>	<b>Page</b>
<b>EUROPEAN FOREWORD</b> .....	<b>5</b>
<b>INTRODUCTION</b> .....	<b>6</b>
<b>1 SCOPE</b> .....	<b>7</b>
<b>2 NORMATIVE REFERENCES</b> .....	<b>7</b>
<b>3 TERMS AND DEFINITIONS</b> .....	<b>7</b>
<b>4 PRINCIPLE</b> .....	<b>9</b>
<b>5 EQUIPMENT AND MATERIALS</b> .....	<b>10</b>
5.1 PRESSURE MEASUREMENT SYSTEM .....	10
<b>FIGURE 1 — PRESSURE MEASUREMENT SYSTEM FOR MEASURING BIODEGRADATION UNDER ANOXIC CONDITIONS (EXAMPLE)</b> .....	<b>11</b>
5.2 STIRRING PLATFORM OR SINGLE MAGNETIC STIRRERS.....	11
5.3 ROOM OR INCUBATOR WITH A CONSTANT TEMPERATURE OF $(20 \pm 2) ^\circ\text{C}$ .....	11
5.4 ARGON FOR THE ELIMINATION OF OXYGEN FROM THE MEDIUM AND THE GAS SPACE.....	11
5.5 THERMOMETER.....	12
5.6 MEMBRANE FILTER .....	12
5.7 ACTIVATED SLUDGE FROM THE DENITRIFICATION TANK OF A WASTE WATER TREATMENT PLANT .....	12
5.8 KOH OR NaOH USED FOR SORPTION .....	12
5.9 HCL USED FOR TITRATION .....	12
5.10 PH METER WITH ELECTRODES .....	12
5.11 CHEMICALS, PIPETTES, PIPETTE TIPS .....	12
5.12 PHOTOMETRIC CUVETTE TESTS.....	12
5.13 PHOTOMETER FOR THE PROCEDURES OR CUVETTE TESTS .....	13
5.14 ANALYTICAL BALANCE .....	13
<b>6 PREPARATION</b> .....	<b>13</b>
6.1 DETERMINATION OF THE VOLUME OF EACH REACTION VESSEL .....	13
6.2 SAMPLE PREPARATION .....	13
6.3 PREPARATION OF THE MEDIUM.....	13
6.3.1 <i>Reagents</i> .....	13
6.3.2 <i>Distilled or deionized water</i> .....	13
6.3.3 <i>Preparation of the concentrates</i> .....	13
6.3.4 <i>Preparation of the medium</i> .....	15
6.4 PREPARATION OF THE INOCULUM.....	15
<b>7 TEST PROCEDURE</b> .....	<b>15</b>
7.1 START OF TEST.....	15
<b>TABLE 1 — EXAMPLE OF ADDITION INTO THE REACTION VESSELS</b> .....	<b>16</b>
7.2 DETERMINATION OF THE INITIAL CONCENTRATIONS (ANALYTICAL SAMPLE) .....	17
7.2.1 <i>Determination of pH</i> .....	17

7.2.2	Determination of the suspended solids of the inoculum .....	17
7.2.3	Determination of ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen (for a nitrogen balance).....	17
7.2.4	Protein determination (for a nitrogen and carbon balance).....	17
7.2.5	Determination of DOC (for a carbon balance).....	17
7.3	INCUBATION PERIOD.....	17
7.4	END OF TEST.....	18
7.5	DETERMINATION OF CO <sub>2</sub> ABSORBED IN ABSORPTION VESSELS (FOR A CARBON BALANCE) .....	18
<b>8</b>	<b>CALCULATION AND EVALUATION.....</b>	<b>18</b>
8.1	CALCULATION OF THE THEORETICAL N <sub>2</sub> PRODUCTION .....	18
8.2	CALCULATION OF THE PRESENT N <sub>2</sub> PRODUCTION .....	19
8.3	CALCULATION OF THE LEVEL OF DEGRADATION RELATED TO THE NITROGEN PRODUCTION .....	20
8.4	GENERATION OF A NITROGEN BALANCE .....	20
<b>TABLE 2 — EXAMPLE OF A NITROGEN BALANCE FOR TESTING THE ANOXIC DEGRADATION WITH PHB AS THE TEST SUBSTANCE (INITIAL MASS: M = 100 MG) .....</b>		
8.5	EVALUATION AND EXPRESSION OF RESULTS .....	21
<b>9</b>	<b>VALIDITY OF RESULTS .....</b>	<b>21</b>
<b>10</b>	<b>TEST REPORT.....</b>	<b>21</b>
<b>ANNEX A (INFORMATIVE) TEST SCHEME — CALCULATION OF THE MAXIMUM PERMITTED INITIAL MASS OF THE TEST SUBSTANCE AND THE MINIMUM NITRATE CONCENTRATION .....</b>		
A.1	GENERAL.....	23
A.2	UPPER PRESSURE MEASUREMENT LIMIT .....	23
A.3	SORPTION CAPACITY OF THE SORPTION SOLUTION (IF APPLICABLE) .....	24
A.4	PH BUFFER CAPACITY OF THE MEDIUM .....	24
A.5	MINIMUM NITRATE CONCENTRATION .....	25
<b>ANNEX B (INFORMATIVE) EXAMPLES OF DEGRADATION CURVES .....</b>		
<b>FIGURE B.1 — PRESSURE INCREASE DURING A BIODEGRADATION TEST UNDER ANOXIC CONDITIONS WITH PHB (POLY-B-HYDROXYBUTYRIC ACID) AND BLANK VALUE .....</b>		
<b>FIGURE B.2 — PERCENTAGE BIODEGRADATION RELATED TO THE NITROGEN PRODUCTION OF A TEST SUBSTANCE (PHB IN THIS CASE) IN A DEGRADATION TEST UNDER ANOXIC CONDITIONS — SAME TEST AS IN FIGURE B.1 .....</b>		
<b>ANNEX C (INFORMATIVE) CALCULATION OF THE PRODUCED INORGANIC CARBON AND PREPARATION OF A CARBON BALANCE .....</b>		
C.1	CALCULATION OF THE PRODUCED INORGANIC CARBON .....	29
<b>FIGURE C.1 — EXAMPLES OF TITRATION CURVES OF THE ORIGINAL KOH (NEW, ORIGINAL, 25 MMOL) AND OF THE SORPTION SOLUTIONS OF A BLANK TEST AND A TEST WITH PHB (100 MG) AFTER COMPLETION OF THE TEST (STANDARD TEST CONDITIONS) .....</b>		
C.2	GENERATION OF A CARBON BALANCE.....	30
<b>TABLE 3 — EXAMPLE OF A CARBON BALANCE FOR PHB (INITIAL MASS: M = 100 MG) .....</b>		
<b>31</b>		

prEN 17417:2019 (E)

C.3 CALCULATION OF THE LEVEL OF BIODEGRADATION RELATED TO CARBON..... 31

BIBLIOGRAPHY..... 32

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## European foreword

This document (prEN 17417:2019) has been prepared by Technical Committee CEN/TC 249 “Plastics”, the secretariat of which is held by NBN.

This document is currently submitted to the CEN Enquiry.

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## Introduction

Biodegradation of chemical substance strongly depends on environmental conditions. The presence or the absence of oxygen is significant for the metabolic pathway on which the degradation of bacteria can take place. At present, several test methods for the investigation of biodegradability of polymers under aerobic conditions, but only a few test methods for the investigation of biodegradability under anaerobic conditions exist. However, degradation under anoxic (denitrifying) conditions has barely been considered yet. The concept "anoxic" has been created by engineers and designates conditions under which denitrification can take place. This means that either a little amount of oxygen or no oxygen at all ( $< 0,1 \text{ mg/l}$ ) but nitrate ( $> 0,1 \text{ mg/l NO}_3^- \text{-N}$ ) is present. During heterotrophic denitrification, e.g. inside the denitrification tank of a wastewater treatment plant, nitrate is reduced to nitrogen and at the same time organic substrate is oxidized to  $\text{CO}_2$ . In nature, anoxic conditions can be present within the hypolimnion of eutrophic lakes or within the sediment at the transition zone between the aerobic and the anaerobic zone.

A way to use biodegradable polymers after intended service life would be their addition as additional carbon source to the denitrification unit of a wastewater treatment plant. In order to check if this way of disposing a polymer is possible, the biodegradability under anoxic (denitrifying) conditions shall be determined. Even if a substance shows good aerobic degradability, this does not necessarily apply under anoxic conditions.

Furthermore, a distinction shall be made between biodegradable polymers that are soluble in water and those not soluble in water.

Those biodegradable polymers that are soluble in water could be added systematically and continuously to the denitrification unit as a solid substrate, which is quickly converted and which can therefore replace the addition of an external liquid carbon source such as ethanol or acetic acid. Testing their aerobic degradability, their water solubility and, if necessary, their water dispersibility can be carried out in accordance with EN 14987. In addition to this, special testing regarding their use as a carbon source for denitrification is done according to this standard. As long as these biodegradable polymers are present as a solid substance, it shall be ensured that they remain in the denitrification tank in order to prevent operational failure during other phases of the wastewater treatment plant.

Those biodegradable polymers that are not soluble in water are discontinuously introduced as a solid substance into a specially designed denitrification reactor, where they substantially remain because of an appropriate process control. Induced by bacterial activity, they continuously release carbon for the purpose of denitrification during a process of anoxic degradation, the duration of which depends on their dimensions (surface/volume ratio). Special testing regarding their use as a water insoluble carbon source for denitrification is described in this standard.



## 1 Scope

This document specifies a method for the determination of the ultimate anoxic biodegradability of plastics made of organic compounds, where the amount of the produced nitrogen and carbon dioxide at the end of the test is measured.

The test substance is exposed to an inoculum stemming from the denitrification tank of a wastewater treatment plant. Testing is performed under defined laboratory conditions.

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 872:2005, *Water quality — Determination of suspended solids - Method by filtration through glass fibre filters*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

### 3.1

#### **ultimate anoxic biodegradation**

degradation of an organic compound into carbon dioxide, water and mineral salts of any of the present elements (mineralization) as well as new biomass by means of microorganisms in the presence of oxidized nitrogen compounds (nitrate, nitrite) and in the absence of oxygen

### 3.2

#### **suspended solids**

solids removed by filtration under specified conditions

[SOURCE: EN 872:2005, 3.1]

### 3.3

#### **dissolved inorganic carbon**

##### **DIC**

that part of the inorganic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at  $40\,000\text{ m s}^{-2}$  for 15 min or by membrane filtration using membranes with pores of  $0,2\ \mu\text{m}$  to  $0,45\ \mu\text{m}$  in diameter

[SOURCE: EN ISO 14852:2004, 3.4] [1]

### 3.4

#### **lag phase**

time, measured in days, from the start of a test until adaptation and/or selection of the degrading microorganisms is achieved and the level of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation

**prEN 17417:2019 (E)**

[SOURCE: EN ISO 14855-1:2013, 3.7] [2]

**3.5****level of biodegradation related to the nitrogen production**

measured level of biodegradation of a chemical compound or organic substance in a test, calculated from the amount of actually produced nitrogen divided by the theoretical maximum amount of nitrogen

Note 1 to entry: It is expressed as a percentage.

**3.6****level of biodegradation related to carbon**

measured level of biodegradation of a chemical compound or organic substance in a test, calculated from the final products of mineralization of the carbon fraction (amount of carbon from carbon dioxide and biomass) divided by the carbon fraction of the amount of the test substance used

Note 1 to entry: It is expressed as a percentage.

**3.7****maximum level of biodegradation**

measured level of biodegradation of a chemical compound or organic substance in a test, above which no further biodegradation takes places during the test

Note 1 to entry: It is expressed as a percentage.

[SOURCE: EN ISO 14855-1:2013, 3.8, modified — the unit “percentage” has been included in the Note] [2]

**3.8****biodegradation phase**

time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

Note 1 to entry: It is expressed in days.

[SOURCE: EN ISO 14855-1:2013, 3.9, modified — the unit “days” has been included in the Note] [2]

**3.9****plateau phase**

time from the end of the biodegradation phase until the end of a test

Note 1 to entry: It is expressed in days.

[SOURCE: EN ISO 14855-1:2013, 3.10, modified — the unit “days” has been included in the Note] [2]

**3.10****nitrogen recovery rate**

sum of the mass concentrations of the nitrogen fractions of nitrate, nitrite, ammonium, protein and of elementary nitrogen at the end of the test divided by the sum of the corresponding mass concentrations at the beginning of the test

Note 1 to entry: It is expressed in percent.

**3.11****carbon recovery rate**

sum of the mass concentrations of the dissolved organic carbon (DOC), the carbon fractions of the test substance, of carbon dioxide and of biomass at the end of the test divided by the sum of the corresponding mass concentrations at the beginning of the test

Note 1 to entry: It is expressed in percent.

**3.12****theoretical oxygen demand****ThOD**

theoretical maximum amount of oxygen required to completely oxidize a chemical compound

Note 1 to entry: It is calculated from the molecular formula of this compound and expressed in milligram of oxygen uptake per milligram of the test compound.

**3.13****theoretical nitrogen production****ThNP**

theoretical maximum amount of nitrogen produced during biodegradation under denitrifying conditions

Note 1 to entry: It is calculated from the molecular formula of the test compound and the stoichiometry of the anoxic biodegradation of this compound (simplified, without biomass) and expressed in milligram of nitrogen produced per milligram of the test compound.

**3.14****theoretical nitrate demand****ThND**

theoretical maximum amount of nitrate-nitrogen that is reduced during biodegradation under denitrifying conditions

Note 1 to entry: It is calculated from the molecular formula of the test compound and the stoichiometry of the anoxic biodegradation of this compound (simplified, without biomass) and is expressed in milligram of nitrate-nitrogen produced per milligram of the test compound.

**3.15****preadaptation**

pre-incubation of an inoculum in the presence of the chemical compound or organic substance under test, with the aim of enhancing the ability of the inoculum to biodegrade the test substance by adaptation and/or selection of the microorganisms

**4 Principle**

In order to investigate anoxic degradation, the nitrogen production ( $N_2$ ) is monitored by means of pressure measurement in a closed system.

A mineral salt medium, free of oxygen and containing nitrate, with the test substance being the only carbon source, is placed in a pressure-tight bottle and inoculated with the inoculum from the denitrification tank of a waste water treatment plant; The gas space of the bottle shall be gassed with argon or nitrogen in order to prevent oxygen from entering. Subsequently, the vessel is closed by means of a pressure measuring head. The use of KOH or NaOH is favourable if the absorbed  $CO_2$  is to be measured by means of titration in order to prepare a carbon balance.

Therefore, the pressure increase inside the bottle is proportional to the nitrogen that is produced during denitrification. Pressure measurement values of a sample are captured and recorded by pressure sensors