

Biodegradation Studies and Experiments for Materials in the Marine Environment Series

Part 2: Status of Current ASTM / ISO Standards, Specifications and Research Studies in the Marine Environment

Prof. dr. Linda Amaral-Zettler
Research Leader
[NIOZ Royal Netherlands Institute for Sea Research](#)



Amaral-Zettler Research Group



MBL,
Woods Hole, MA
USA



2017 -present



2019-present



University of Amsterdam



Science & Environment

Ocean plastic a 'planetary crisis' - UN

By Roger Harrabin
BBC environment analyst, Nairobi

9 hours ago | Science & Environment

f t m e Share



Plastic waste has a variety of detrimental effects on the environment

Life in the seas risks irreparable damage from a rising tide of plastic waste, the UN oceans chief has warned.



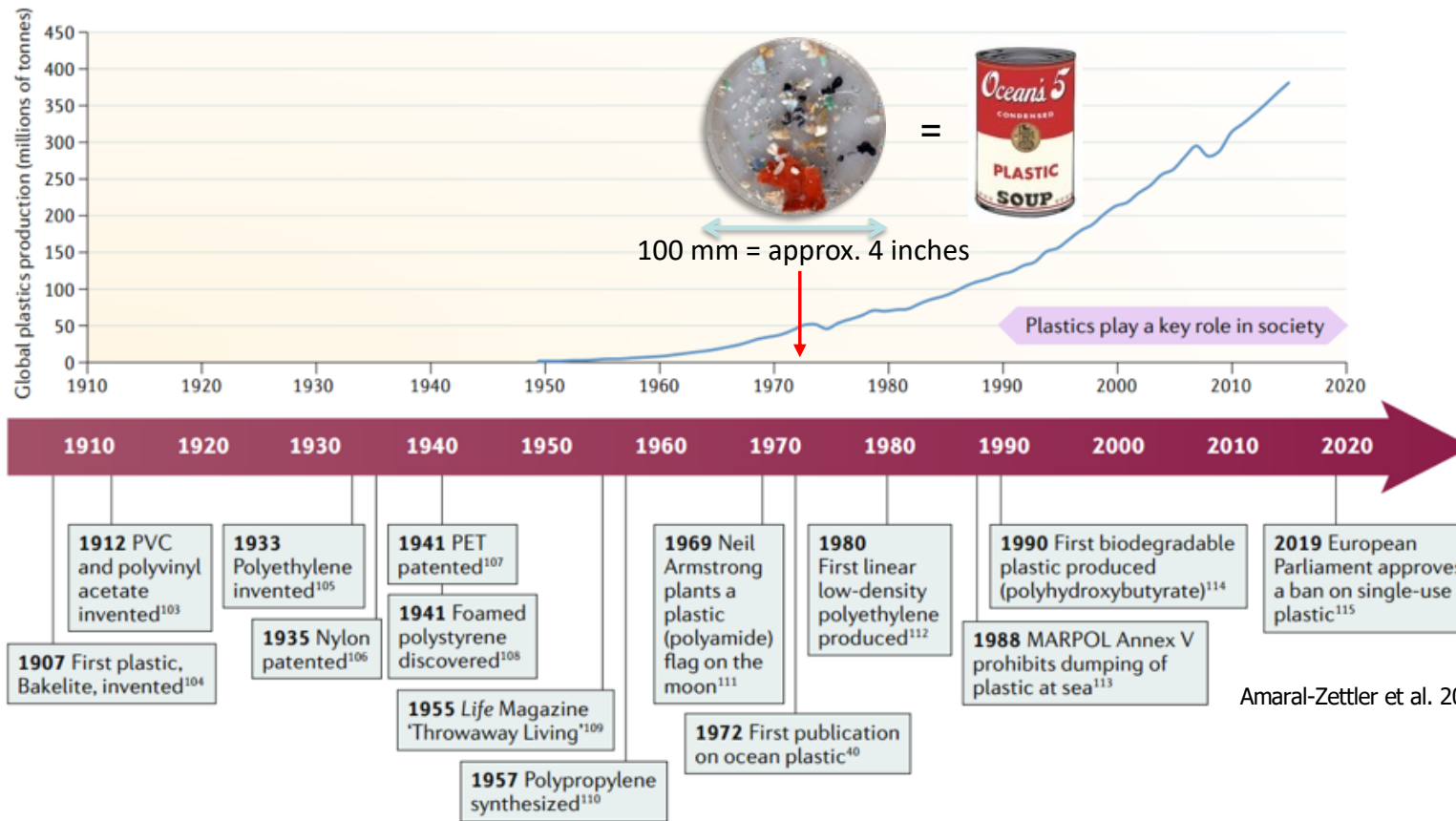
Striking images show plastic litter in the world's most remote coral reefs

Fishing nets, packaging and other debris threaten wildlife in some of the ocean's deepest reef ecosystems.

[Katharine Sanderson](#)



Plastics Timeline



Amaral-Zettler et al. 2020, Nat. Rev. Microbiol.

Approximately 10 million tonnes per year enters ocean

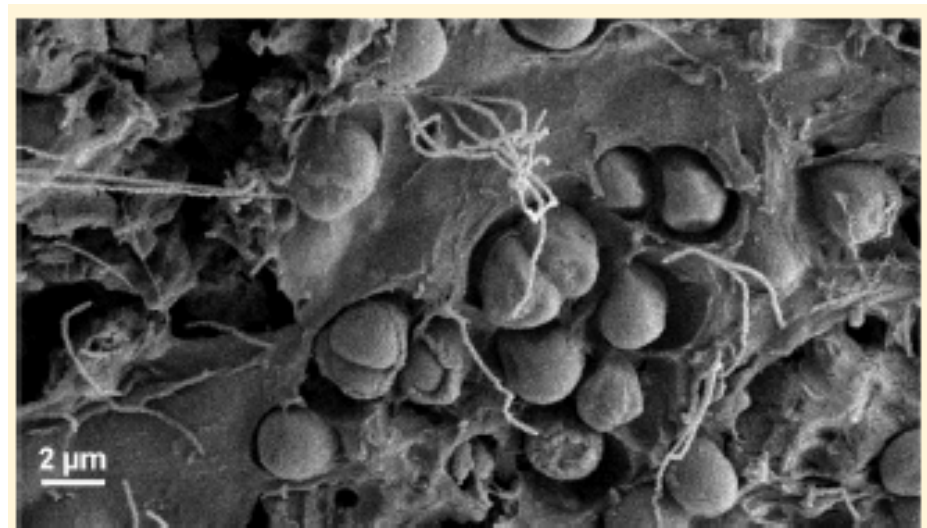
(Jambeck et al. 2015, Science)

Published online 28 March 2011 | Nature | doi:10.1038/news.2011.191

News

Marine microbes digest plastic

A 'little world' eating ocean garbage might be a mixed blessing.



June 2011

Scientific American

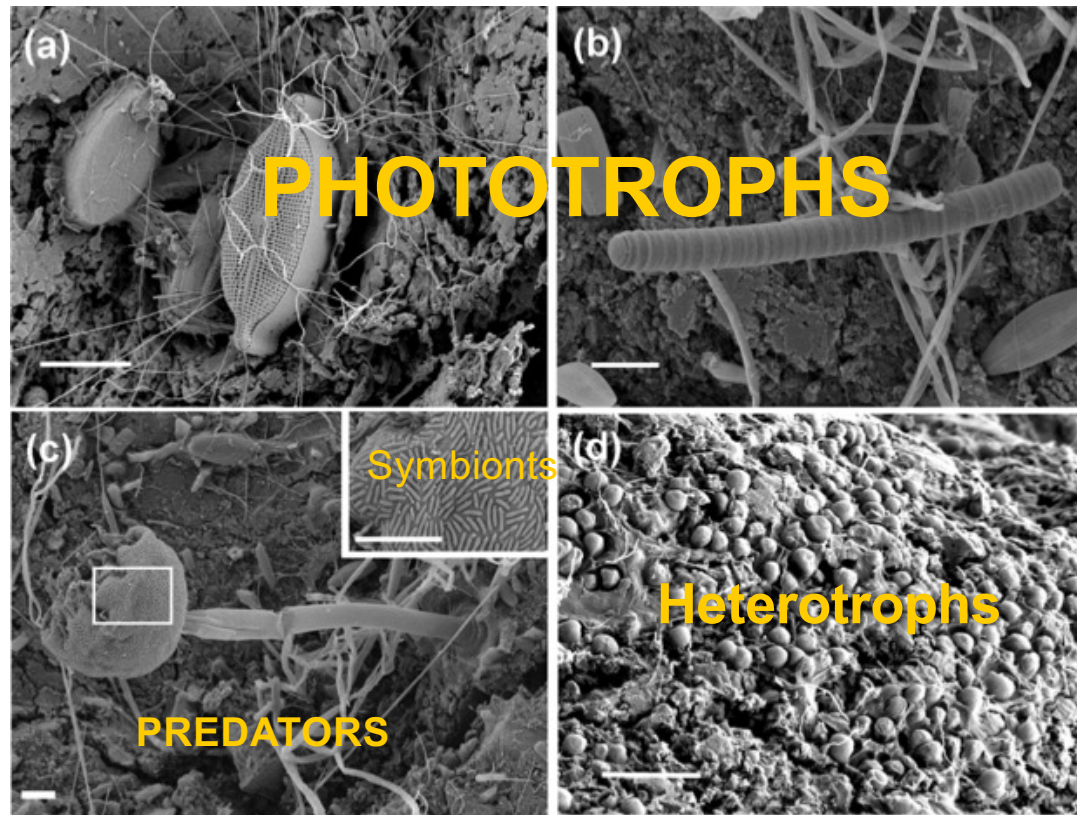
MARINE BIOLOGY

The Smallest Hitchhikers

Marine microbes may hold the key to the ocean's disappearing plastic

Microbial Communities on Plastic Marine Debris: The “Plastisphere”

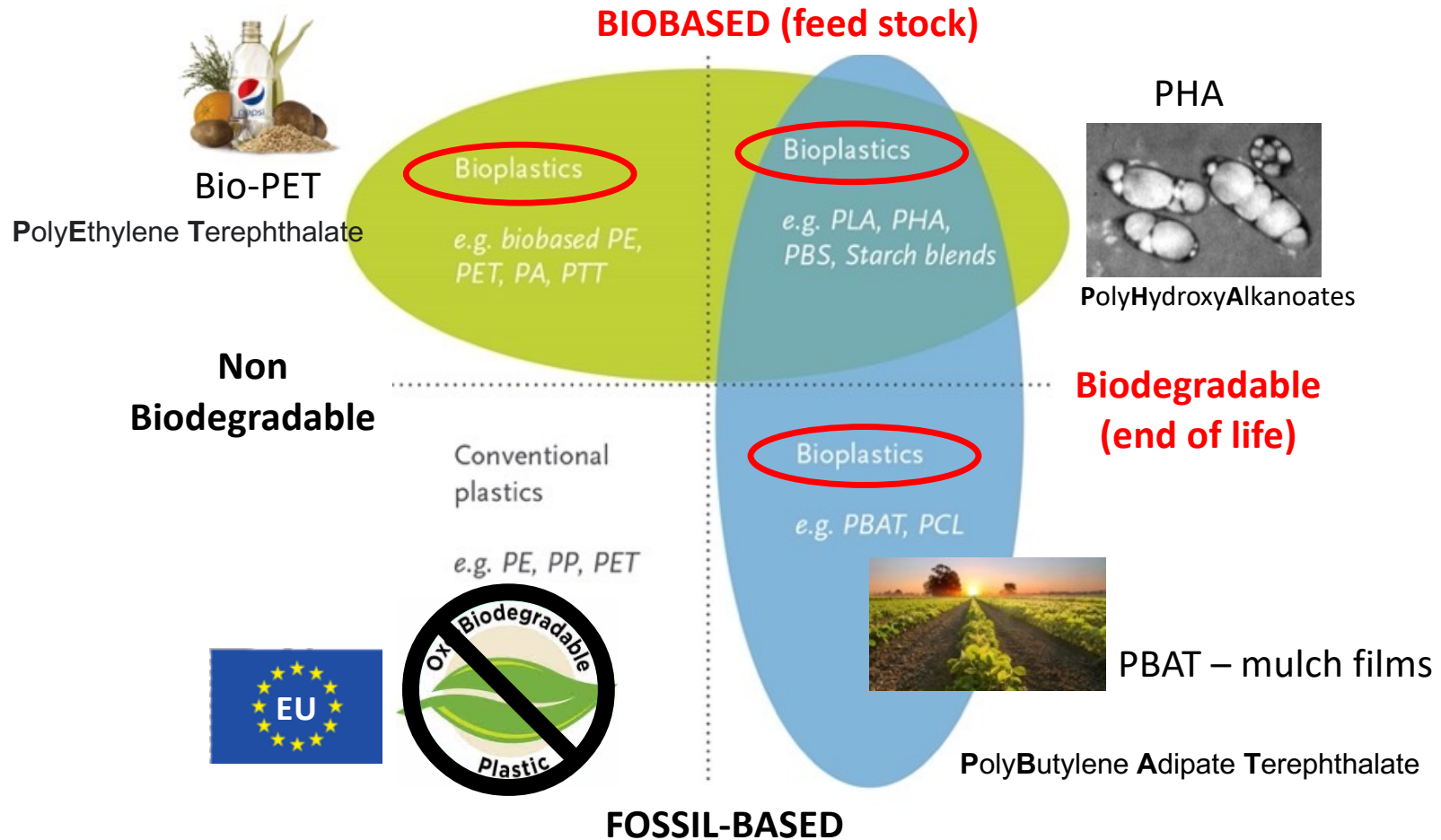
“Like the biosphere (the thin film of life around the surface of planet Earth), the Plastisphere represents a little world of life that exists on the surface of plastic particles”



All scale bars = 10 μm

Zettler, Mincer, Amaral-Zettler 2013

A Bioplastics Solution?





Biodegradation in the Marine Environment

Plastic **biodegradation** is the extensive conversion of polymer carbon to CO_2 (under oxic conditions) or CO_2 and CH_4 (under anoxic conditions), and new microbial biomass, over a specific **timeframe**.

SAPEA Evidence Report 8, 2020

Motivation and Funding that fueled initial Biodegradation Testing & Research



Jo Ann Ratto



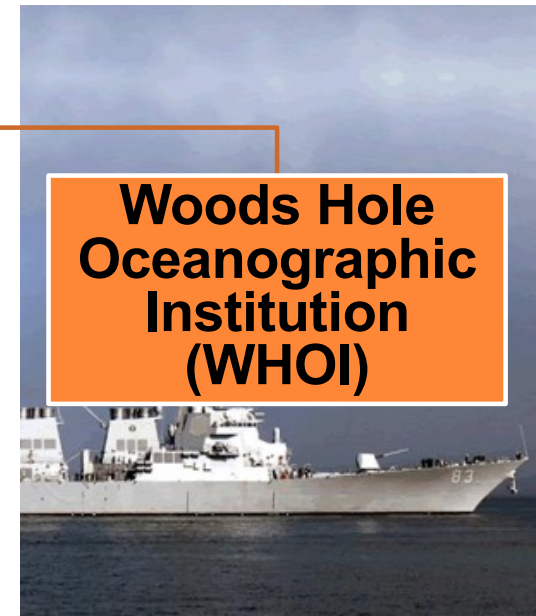
Carl Wirsén



**Biodegradable
Polymer Testing
and
Research**

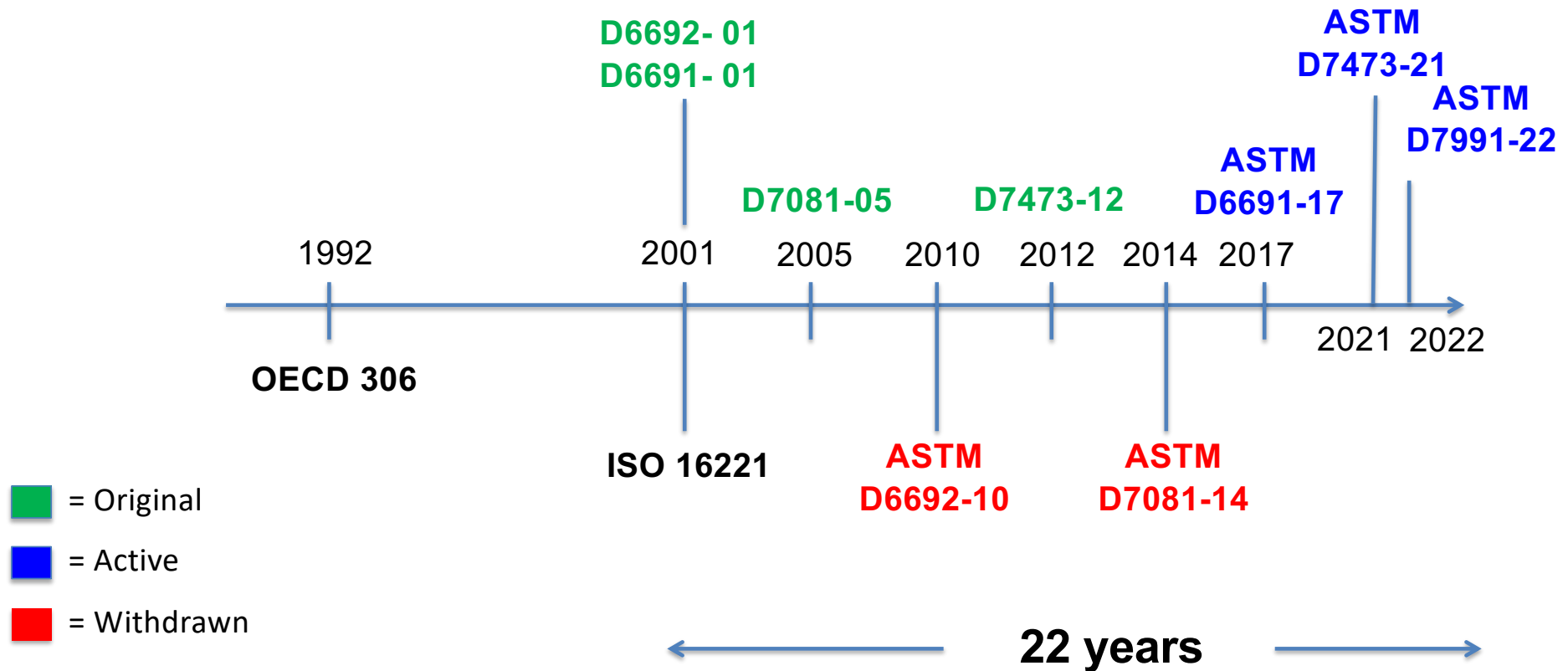


U.S. Army



Courtesy of Jo Ann Ratto
UNCLASSIFIED

A Timeline of Biodegradation Methods in the Marine Environment



ASTM Standards Terminology

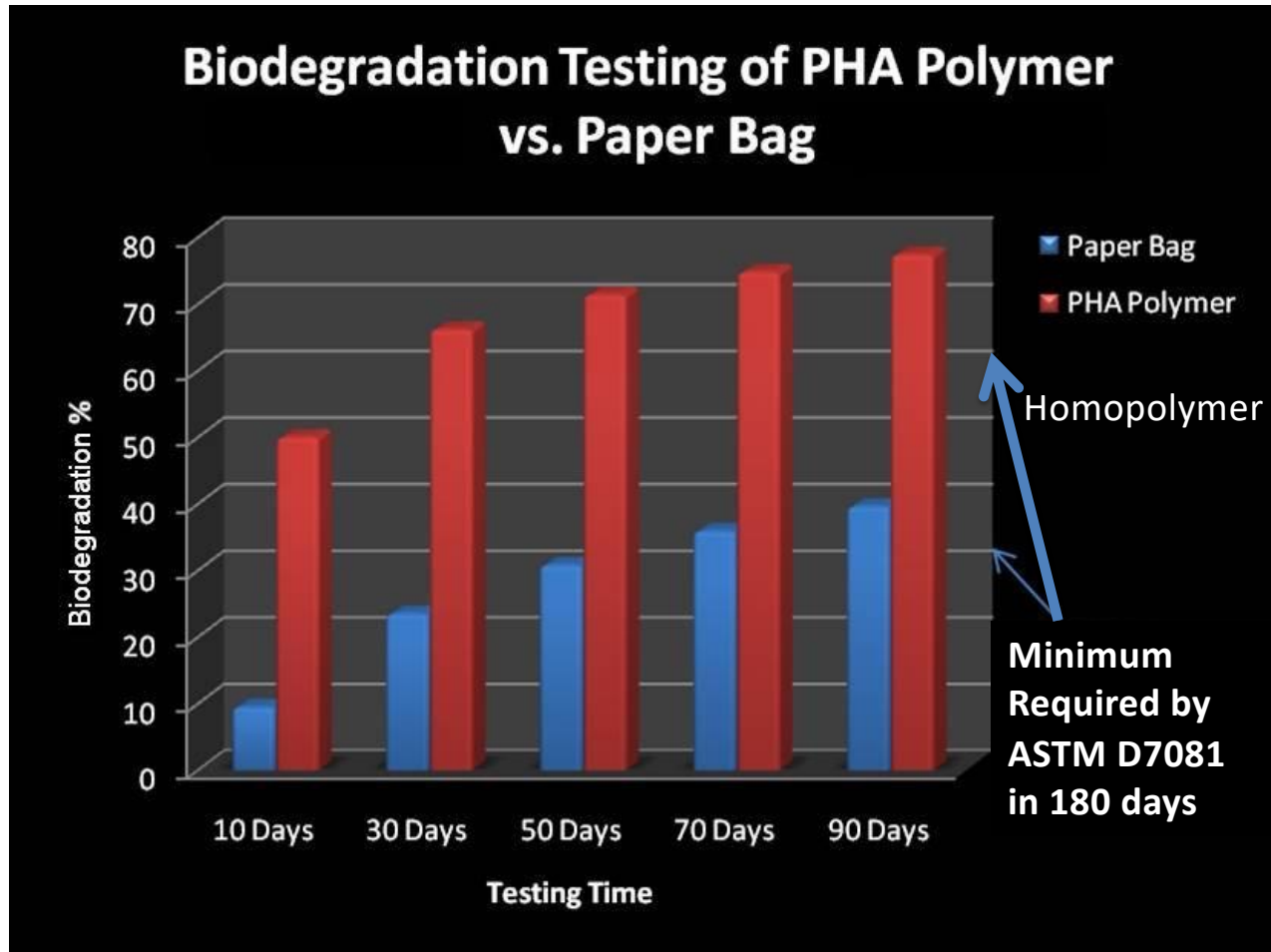
1. **Standard Practice** [**practice, n**— a definitive set of instructions for performing one or more specific operations that does not produce a test result – ASTM Bluebook April, 2016]
2. **Standard test method** [**test method, n**— a definitive procedure that produces a test result -- ASTM Bluebook April, 2016]
3. **Specification** [**specification, n**— an explicit set of requirements to be satisfied by a material, product, system, or service – ASTM Bluebook, April 2016]

- Tier I – Standard methods in laboratory
 - Respirometry methods
 - **ASTM D6691**
 - Standard **Test Method** for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum

- Tier II – Confirmatory in Marine Environment
 - Incubation methods / Weight loss as a function of time
 - Static Laboratory
 - Dynamic Aquarium
 - **ASTM D7473 - Test Method** For Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations

- Tier III – Confirmatory in Marine Environment
 - Incubation methods / Weight loss as function of time
 - Coastal Studies
 - Deep Sea Moorings

Standard Specification D7081 (pass/fail criterion):
Polymer vs. Kraft Paper (Tier I)





ASTM INTERNATIONAL

ASTM International Collaboration Area

Specification for Non-Floating Biodegradable Products in the Aquatic Environment – Replacement for **D7081**

Current Collaboration Area

75797 - WK75797 - Non-Floating Biodegrad...

75797 - WK75797 - Non-Floating Biodegradable Products in the Marine Environment

Collaboration Area

Drafts

Polls

Discussions

Files

Overview

Members

History

WK75797 - Non-Floating Biodegradable Products in the Marine Environment

Group Creation Date: 02/08/2021

WorkItem Creation Date: 02/08/2021

Ballot Target Date: 12/2023

Work Item Status: Proposed

Status: Draft Withdrawn

Work Item Description

There is an industry need for a standardized approach to demonstrate product conformance for biodegradability in a marine environment. Currently, with no active ASTM specification standard, state and organizations are developing their own methods or prohibiting the use of terminology that implies biodegradation in a marine environment. The need to reestablish a specification standard in this area, with a cooperative industry approach, is critical. Users will include, academia, testing labs, government and industry manufacturing.



ASTM INTERNATIONAL

ASTM International Collaboration Area

Specification for Non-Floating Biodegradable Products in the Aquatic Environment – Replacement for **D7081**

Current Collaboration Area

75797 - WK75797 - Non-Floating Biodegrad...

Aquatic

75797 - WK75797 - Non-Floating Biodegradable Products in the Marine Environment

Collaboration Area

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ASTM INTERNATIONAL

Standard Specification for Non-Floating Biodegradable Products Designed for use in an **Aquatic Environment**¹

This standard is issued under the fixed designation DXXXXX; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This specification covers non-floating products that are designed for use in an aquatic environment and are biodegradable in a stated aquatic environment.

1.2 This specification is intended to establish the requirements for labeling products as “Biodegradable in a *stated aquatic* environment as a non-floating object at 20°C in X , per ASTM DXXXXX.” The label must use qualifying language that states the type of aquatic environment.

NOTE 1- The stated aquatic environment will be based on the testing standard and the inoculum. It is not the intent of this standard to specify the type of aquatic environment.

NOTE 2 - 20°C may be replaced with 68°F if the claim is being made in a country that uses the imperial system.

NOTE 3 - Where “ X ” is the time and can be denoted in either total days or a combination of years and days.

1.3 The properties in this specification are those required to determine if products designed for use in the aquatic environment will biodegrade with specific rate/rate constant in aquatic environments at designated temperature(s).

1.4 This standard does not promote or encourage the disposal or loss or leakage of biodegradable materials or products into the aquatic environment. Nor does it address microplastics, nanoplastics, microparticles or nanoparticles. However, a fully biodegradable material or product will not, by this standard, permanently generate microplastics, nanoplastics, microparticles or nanoparticles. Microplastics, nanoplastics, microparticles or nanoparticles may be generated in the process of the material or product going through full biodegradation, though they will not exist after complete biodegradation.



- Requires biodegradation rates at three different temperatures
- Uses Kinetic Rate Law and Arrhenius Equation
- Used to obtain lifetime predictions of products to achieve 90% biodegradation at a given temperature as a basis for comparative analyses



Revision: D6691

ASTM INTERNATIONAL

ASTM International Collaboration Area

Current Collaboration Area

79317 - WK82370 - Revision of D6691-17 Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum

Collaboration Area [Drafts](#) [Polls](#) [Discussions](#) [Files](#)

[Overview](#) [Members](#) [History](#)

WK82370 - Revision of D6691-17 Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum

WorkItem Creation Date: 06/22/2022

Ballot Target Date: 12/2023

Work Item Status: Draft Under Development

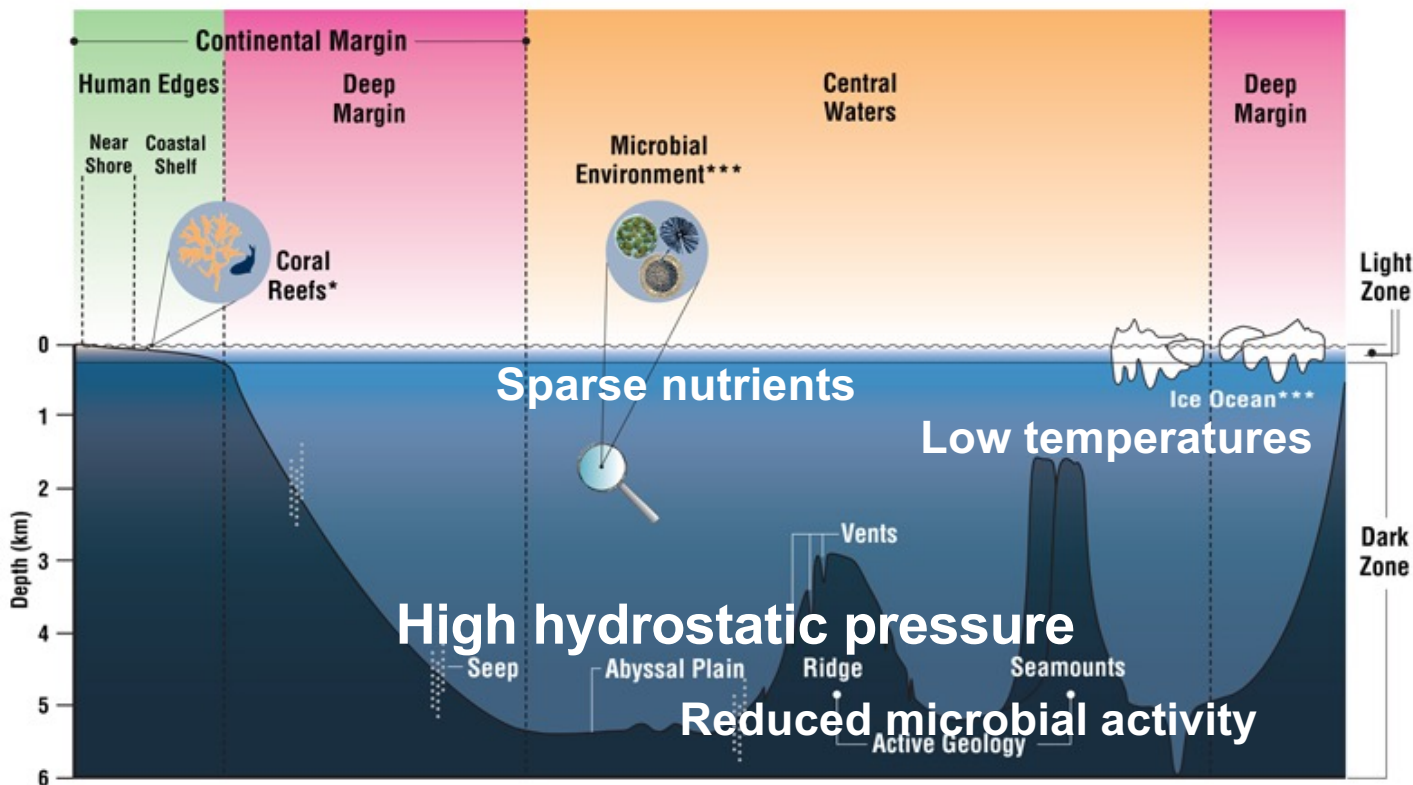
Status: Draft Under Development

Work Item Description

ASTM D6691 is scheduled for review, and committee members have advised that more data is available which might be considered.

The **Marine** Environment is Not Homogeneous

Theoretical Cross Section of the Ocean



* Coral reefs are found in the warm waters of the Atlantic, Pacific, and Indian oceans.

** Microbial environment encompasses the entire world ocean.

*** Ice oceans occur at both poles.

CoML defines its realms & zones in 2003 Baseline Report, *The Unknown Ocean*



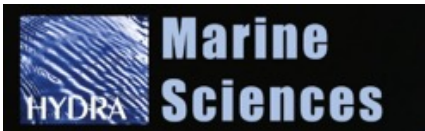
Open Bio: (opening the market for biobased products) Recommendations

EU 7th Framework
Nov 2013-Oct 2016

- Tests under **anaerobic** conditions should be developed
- Tests under **high-pressure** and **low temperature** should be developed
- Tests with fine sediments under **low oxygen** conditions are needed
- Effects of **different levels of nutrients** and **organic contents** should be considered to develop all the standard tests
- Testing in **mesocosms**
- Extract **representative habitats** from nature and translate their conditions to a set of standard laboratory and mesocosm tests to be developed and implemented, representing the **most important regions** of our oceans.

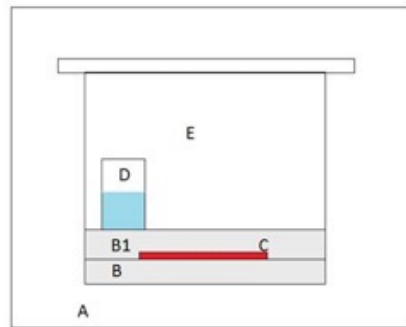


Francesco Degli Innocenti



ASTM D7991-22

“Standard Test Method for Determining Aerobic Biodegradation of Plastics Buried in **Sandy Marine Sediment** under Controlled Laboratory Conditions”





Beat plastic pollution

with ISO standards

ISO 15270
Recovery and recycling
of plastic waste

ISO 22526
Carbon and
environmental footprint

ISO/CD 22766
Disintegration
of plastic materials
in marine habitats

ISO 18830
Biodegradation test



ISO (International Standards Organization): Subcommittee 14: Plastics and the Environment Working Group 2: Biodegradability

ISO/CD 22403, Plastics - Assessment of the inherent aerobic biodegradability and environmental safety of non-floating materials exposed to marine inocula under laboratory and mesophilic conditions - Test methods and requirements

ISO 19679:2016(E) Plastics - Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface - Method by analysis of evolved carbon dioxide

ISO/DIS 23977-1/2 Plastics - Determination of aerobic biodegradation of plastic materials exposed to seawater-

- 1 - Method by analysis of evolved carbon dioxide
- 2 - Method by measuring the oxygen demand in closed respirometer

ISO-TAG Meeting
September 23-28, 2019
Chengdu, China



Marine Standards

	Respirometry / BOD		Weight Loss – Aging / Weathering / Disintegration				
	Laboratory		Aquarium			Field	
Pelagic (Seawater)	ASTM D6691 CO ₂	ISO 23977/1- CO ₂ ISO 23977/2 -O ₂	ISO 15314	ISO/CD 16636	ASTM D7473	ISO 15314	ISO/CD 16636
Pelagic (Seawater/ Seawater + Sediment)	ISO 23977/1- CO ₂ ISO 23977/2 -O ₂						
Benthic (Seafloor)	ISO 18830 O ₂	ISO 19679 CO ₂	ISO 23832		ASTM D7473		
Eulittoral (Beach)	ASTM D7991 CO ₂	ISO 22404 CO ₂	ISO 23832			ISO 22766	

OCEAN PLANKTON

Structure and function of the global ocean microbiome

Shinichi Sunagawa,^{1*†} Luis Pedro Coelho,^{1*} Samuel Chaffron,^{2,3,4*} Jens Roat Kultima,¹ Karine Labadie,⁵ Guillem Salazar,⁶ Bardya Djahanschiri,¹ Georg Zeller,¹ Daniel R. Mende,¹ Adriana Alberti,⁵ Francisco M. Cornejo-Castillo,⁶ Paul I. Costea,¹ Corinne Cruaud,⁵ Francesco d'Ovidio,⁷ Stefan Engelen,⁵ Isabel Ferrera,⁶ Josep M. Gasol,⁶ Lionel Guidi,^{8,9} Falk Hildebrand,¹ Florian Kokoszka,^{10,11} Cyrille Lepoivre,¹² Gipsi Lima-Mendez,^{2,3,4} Julie Poulain,⁵ Bonnie T. Poulos,¹³ Marta Royo-Llonch,⁶ Hugo Sarmento,^{6,14} Sara Vieira-Silva,^{2,3,4} Céline Dimier,^{10,15,16} Marc Picheral,^{8,9} Sarah Seaton,^{8,9} Stefanie Kandels-Lewis,^{1,17} Tara Oceans coordinators† Chris Bowler,¹⁰ Colombar de Vargas,^{15,16} Gabriel Gorsky,^{8,9} Nigel Grimsley,^{18,19} Pascal Hingamp,¹² Daniele Iudicone,²⁰ Olivier Jaillon,^{5,21,22} Fabrice Not,^{15,16} Hiroyuki Ogata,²³ Stephane Pesant,^{24,25} Sabrina Speich,^{26,27} Lars Stemmann,^{8,9} Matthew B. Sullivan,^{13§} Jean Weissenbach,^{5,21,22} Patrick Wincker,^{5,21,22} Eric Karsenti,^{10,17,†} Jeroen Raes,^{2,3,4,†} Silvia G. Acinas,^{8,†} Peer Bork^{1,28,†}

Microbes are dominant drivers of biogeochemical processes, yet drawing a global picture of functional diversity, microbial community structure, and their ecological determinants remains a grand challenge. We analyzed 7.2 terabases of metagenomic data from 243 Tara Oceans samples from 68 locations in epipelagic and mesopelagic waters across the globe to generate an ocean microbial reference gene catalog with >40 million nonredundant, mostly novel sequences from viruses, prokaryotes, and picoeukaryotes. Using 139 prokaryote-enriched samples, containing >35,000 species, we show vertical stratification with epipelagic community composition mostly driven by temperature rather than other environmental factors or geography. We identify ocean microbial core functionality and reveal that >73% of its abundance is shared with the human gut microbiome despite the physicochemical differences between these two ecosystems.

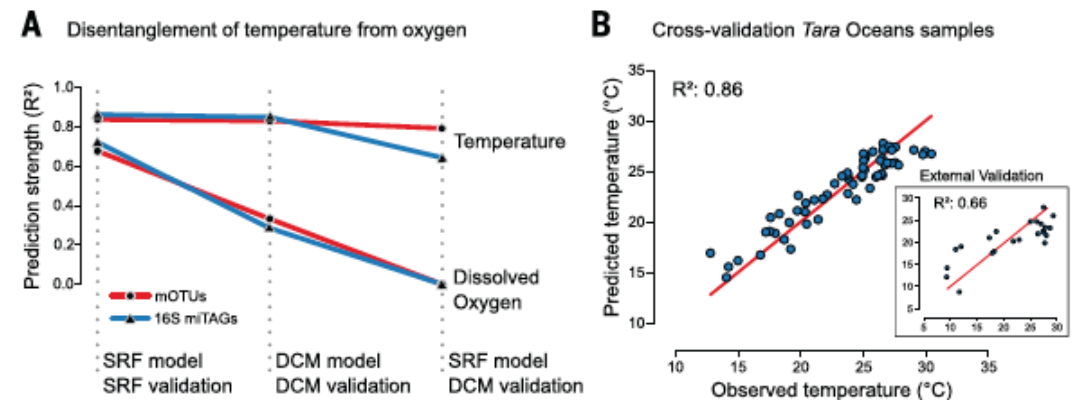
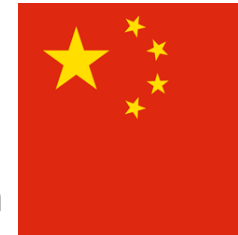
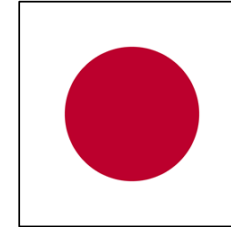


Fig. 6. Temperature as main environmental driver for microbial community composition in the epipelagic layer. (A) The strength of association between (meta)genomic and environmental data was tested by statistical models that were first generated with a subset of data for training and then validated

ISO Round Robin Participants

ISO/DIS 23977-1/2 Plastics

Determination of aerobic biodegradation of plastic materials exposed to seawater



BASF Germany – lead (Chong Becker)
Novamont, Italy
Yahata-Bussan Co., Ltd. Japan

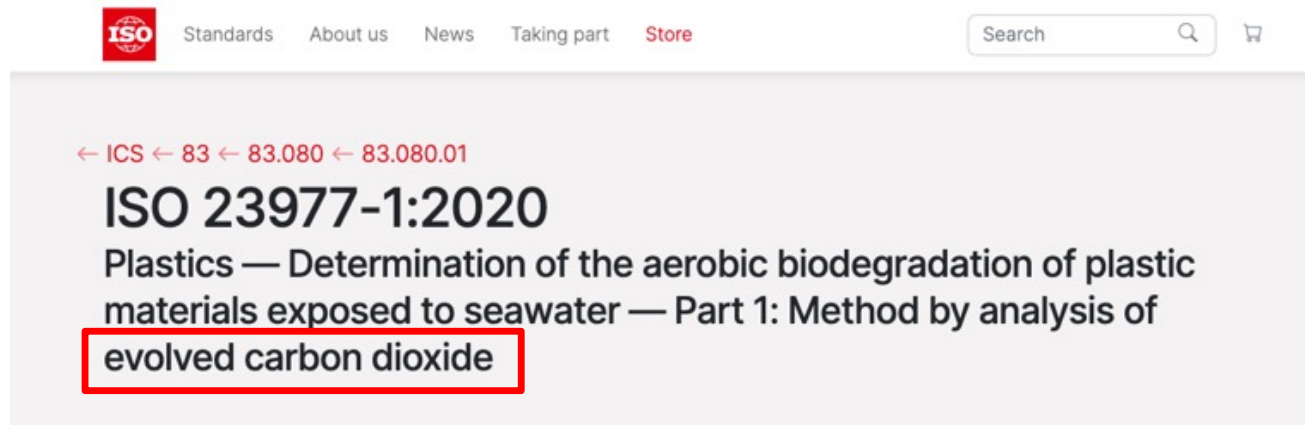
Chemicals Evaluation and Research Institute, Japan
NTSQP, Beijing Technology and Business University

NIOZ Royal Netherlands Institute for Sea Research

Eastman, USA
OWS, Belgium

ISO 22403 Specification Requirements:

- 90 % absolute biodegradation or relative to reference material
- Test duration: Max. 24 months
- Temperature: 15 – 25 ° C (not exceeding 28 ° C)



ISO logo Standards About us News Taking part Store Search

← ICS ← 83 ← 83.080 ← 83.080.01

ISO 23977-1:2020

Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1: Method by analysis of evolved carbon dioxide

This screenshot shows the ISO website page for standard ISO 23977-1:2020. The page title is "ISO 23977-1:2020" and the description is "Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1: Method by analysis of evolved carbon dioxide". The phrase "evolved carbon dioxide" is highlighted with a red box. The navigation bar includes the ISO logo, "Standards", "About us", "News", "Taking part", and "Store" links, along with a search bar and a shopping cart icon.



ISO logo Standards About us News Taking part Store Search

← ICS ← 83 ← 83.080 ← 83.080.01

ISO 23977-2:2020

Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 2: Method by measuring the oxygen demand in closed respirometer

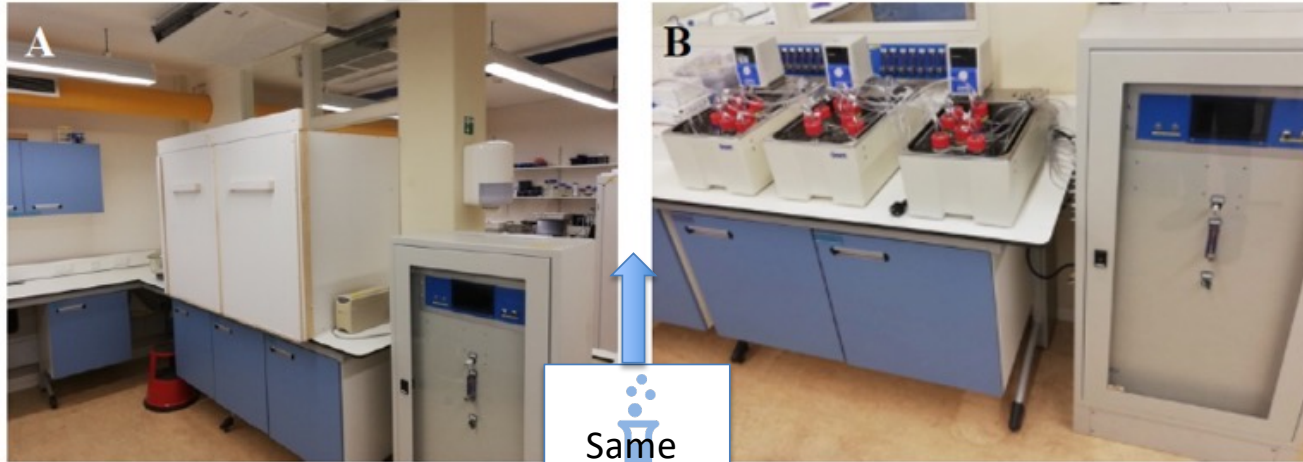
This screenshot shows the ISO website page for standard ISO 23977-2:2020. The page title is "ISO 23977-2:2020" and the description is "Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 2: Method by measuring the oxygen demand in closed respirometer". The phrase "oxygen demand" is highlighted with a red box. The navigation bar includes the ISO logo, "Standards", "About us", "News", "Taking part", and "Store" links, along with a search bar and a shopping cart icon.



ISO/DIS 23977-1/2
Round Robin Test

Open System

Biodegradation
(CO₂ & O₂ infrared)



Same
Inoculum

Closed System

Biodegradation
(BOD & evolved CO₂
Via DIC measurement)



Parallel System

No Biodegradation
monitoring
(Weekly sampling for
Microbial DNA Profiling
& Microscopy)

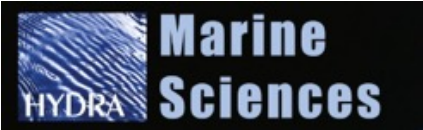




Field and mesocosm test methods to assess the performance of biodegradable plastic under marine conditions

Christian Lott, Andreas Eich, Boris Unger, Dorothee Makarow, Glauco Battagliarin, Katharina Schlegel, Markus T. Lasut, Miriam Weber

doi: <https://doi.org/10.1101/2020.01.31.928606>



Eulittoral

1

PBSeT: PolyButylene Sebacate co butylenTerephthalate

PHA: Polyhydroxyalkanoate Copolymer

LDPE: Low Density Polyethylene

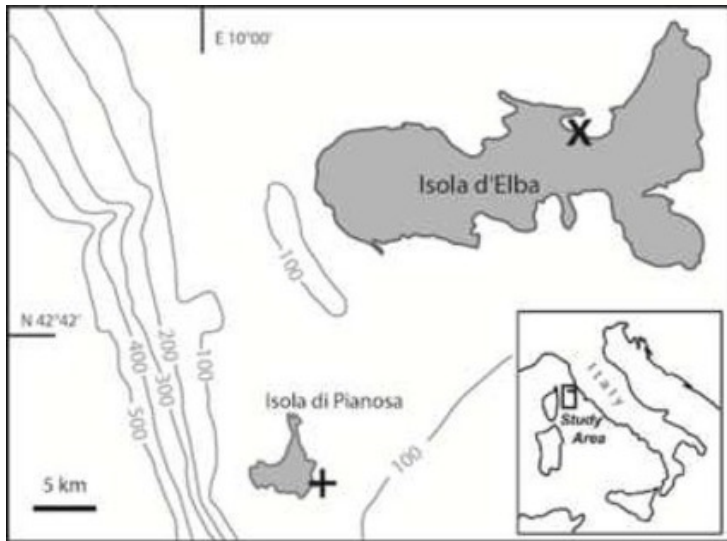
2 Pelagic

3 Benthic

HYDRA 2019

Lott et al. 2020. Field and mesocosm test methods...

Open-Bio design





Elba Sediment and Water

Date of collection: July 6, 2020

Depth of collection (m): Mid water line at the Fetovaia Beach on Elba, Italy

Appearance of Seawater sample – (turbid, clear etc.): Clear

Temperature (°C): 25 °C

Salinity (PSU): 38.5

TOC: 1.9 mg/L

Total-N: 12.6 mg/L (after nutrient addition)

pH: 8.15

No pre-conditioning process applied



OxiTop – Closed System – 12 bottles

Test material	Nr. of replicates
PHBH	3
PE-LD (- control RRT)	3
Cellulose filter (+ control RRT)	3
Blank	3
Total	12



PHBH: copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate) - 25-30 μm

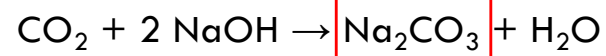
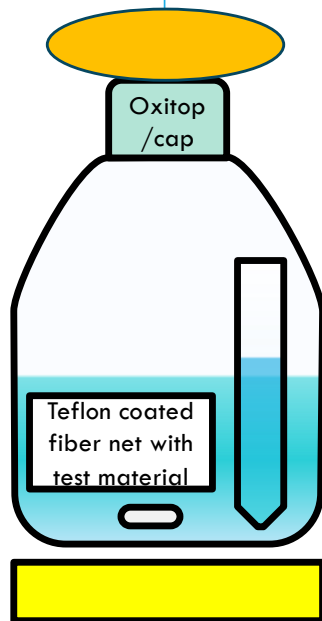
LDPE: low density polyethylene - 25-30 μm

Cellulose Filter Paper: Blauband NEU filter paper - 170 μm

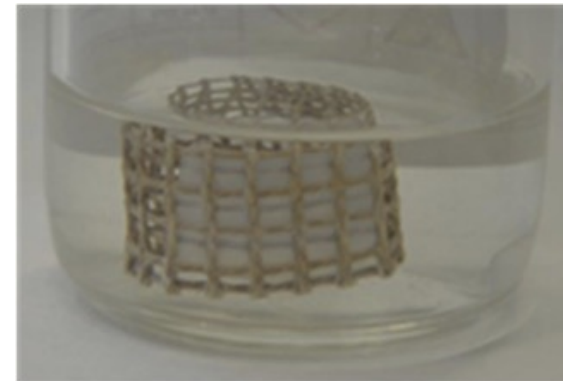
OxiTop – Closed System

Determine Biological Oxygen Demand
via

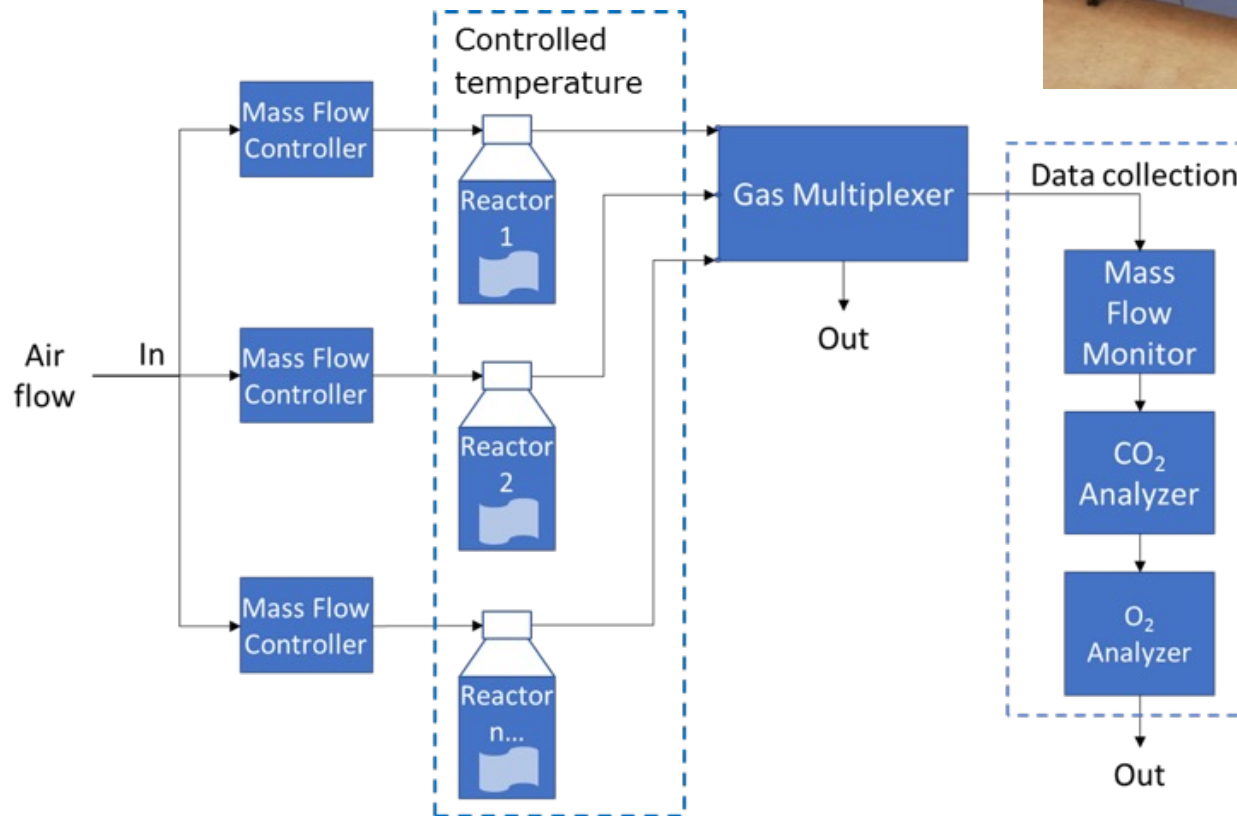
Oxygen Consumption



Measure total Dissolved
Inorganic Carbon as a proxy for CO₂
Evolution



Respirometry – Open System – 20 bottles



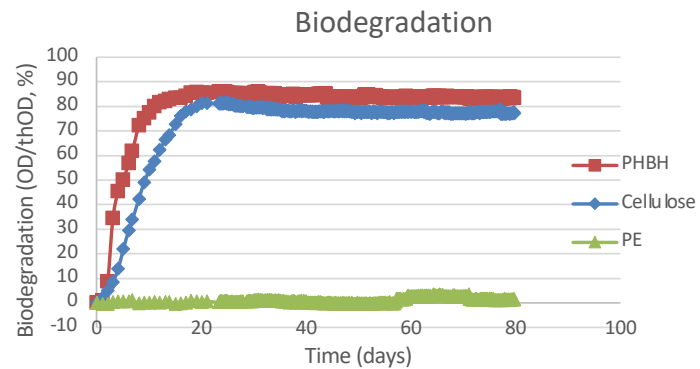
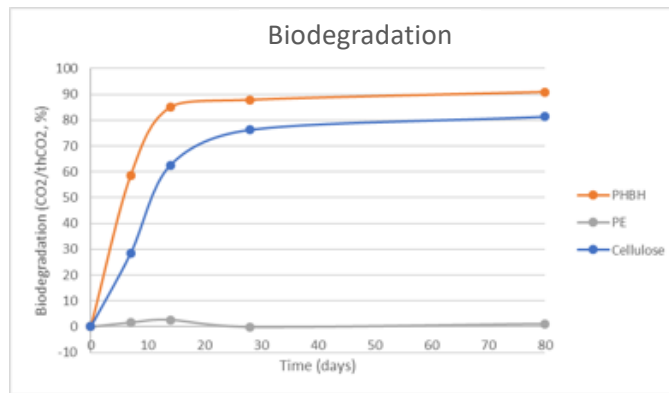
Parallel Plasticsphere Sampling Bottles – 48 bottles

Test material	Nr. of replicates
PHBH	39
PE-LD (- control RRT)	3
Cellulose filter (+ control RRT)	3
Blank	3
Total	48

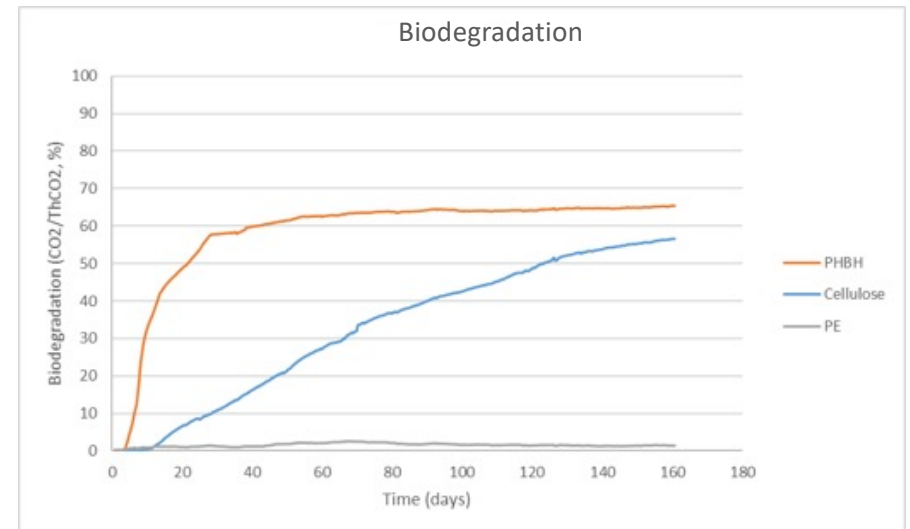


ISO Round Robin Test Results

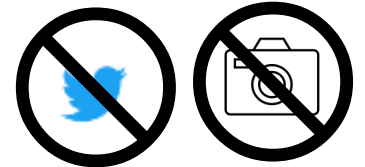
Closed System



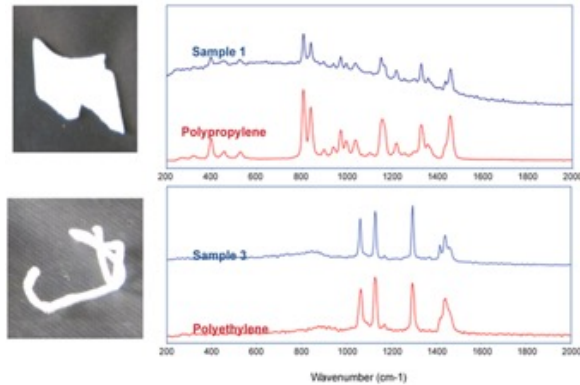
Open System



Amaral-Zettler, unpublished



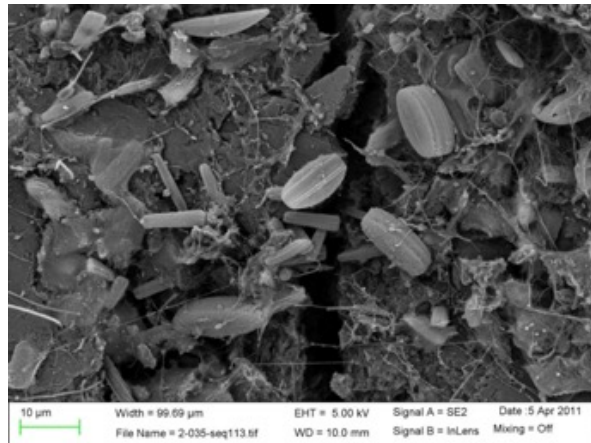
Plastic identification, microscopy, molecular tools, and culturing



Raman / FT-IR Spectroscopy



Illumina Next-Gen Sequencing



Scanning Electron Microscopy

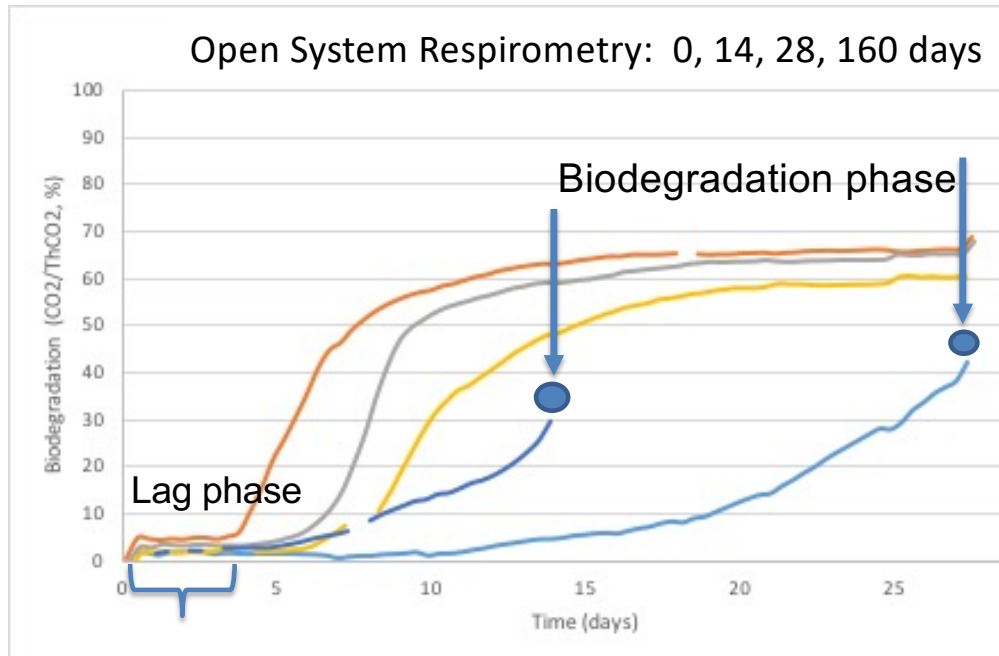


Culture Isolation

Sampling For Microbial Community Profiling

Closed System OxiTop: 0, 80 days

Open System Respirometry: 0, 14, 28, 160 days



Plastisphere:
7, 14, 21, 28 & 93 days



BIOLOGY

SEM FISH Who?

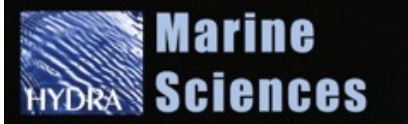
Amplicon Sequencing Where?

Metagenomics/ Metatranscriptomics What?

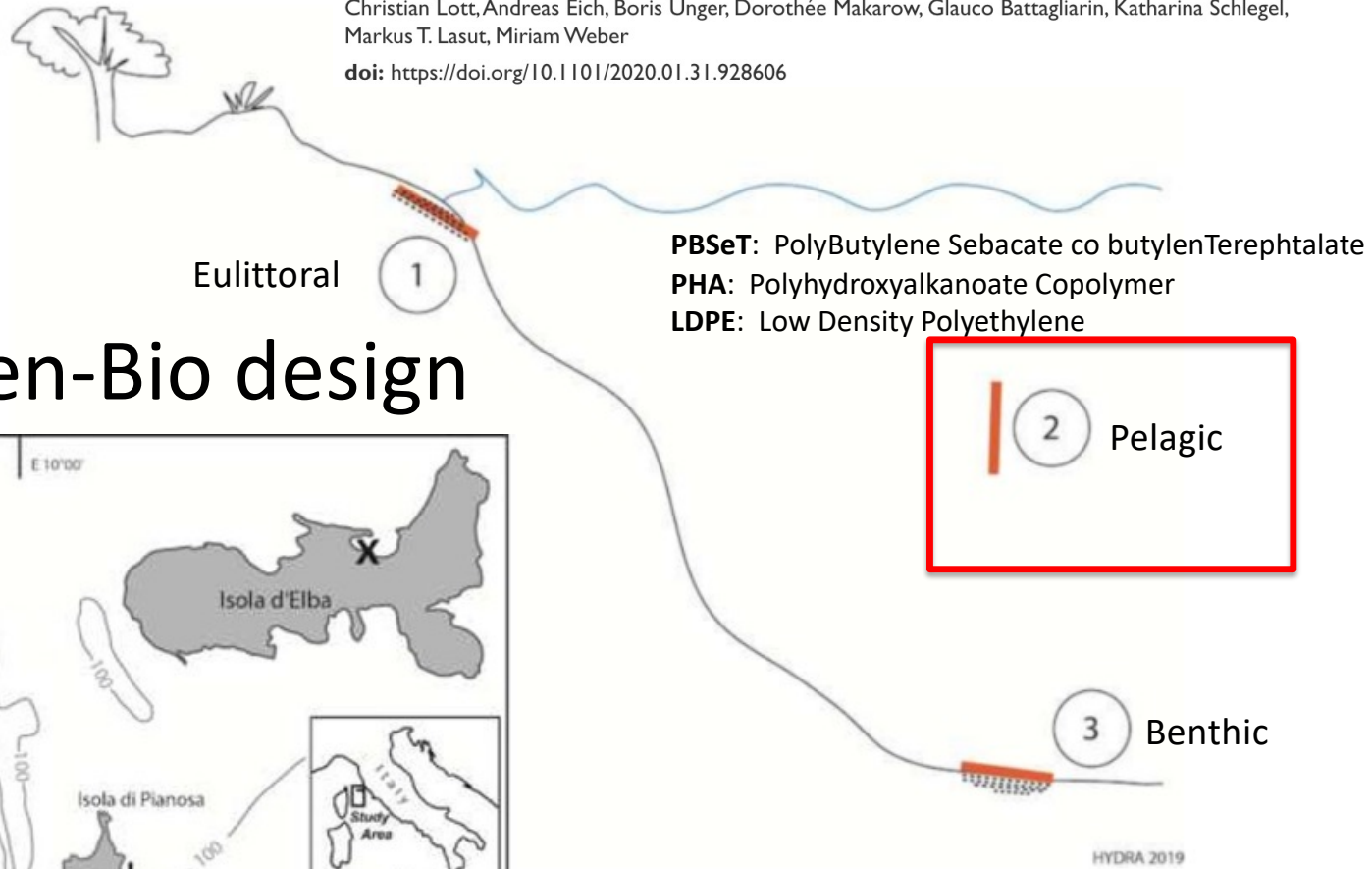
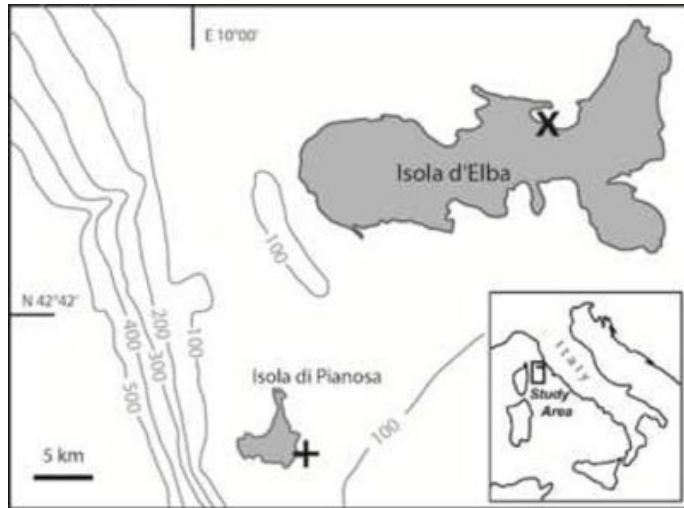
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doi: <https://doi.org/10.1101/2020.01.31.928606>



Open-Bio design

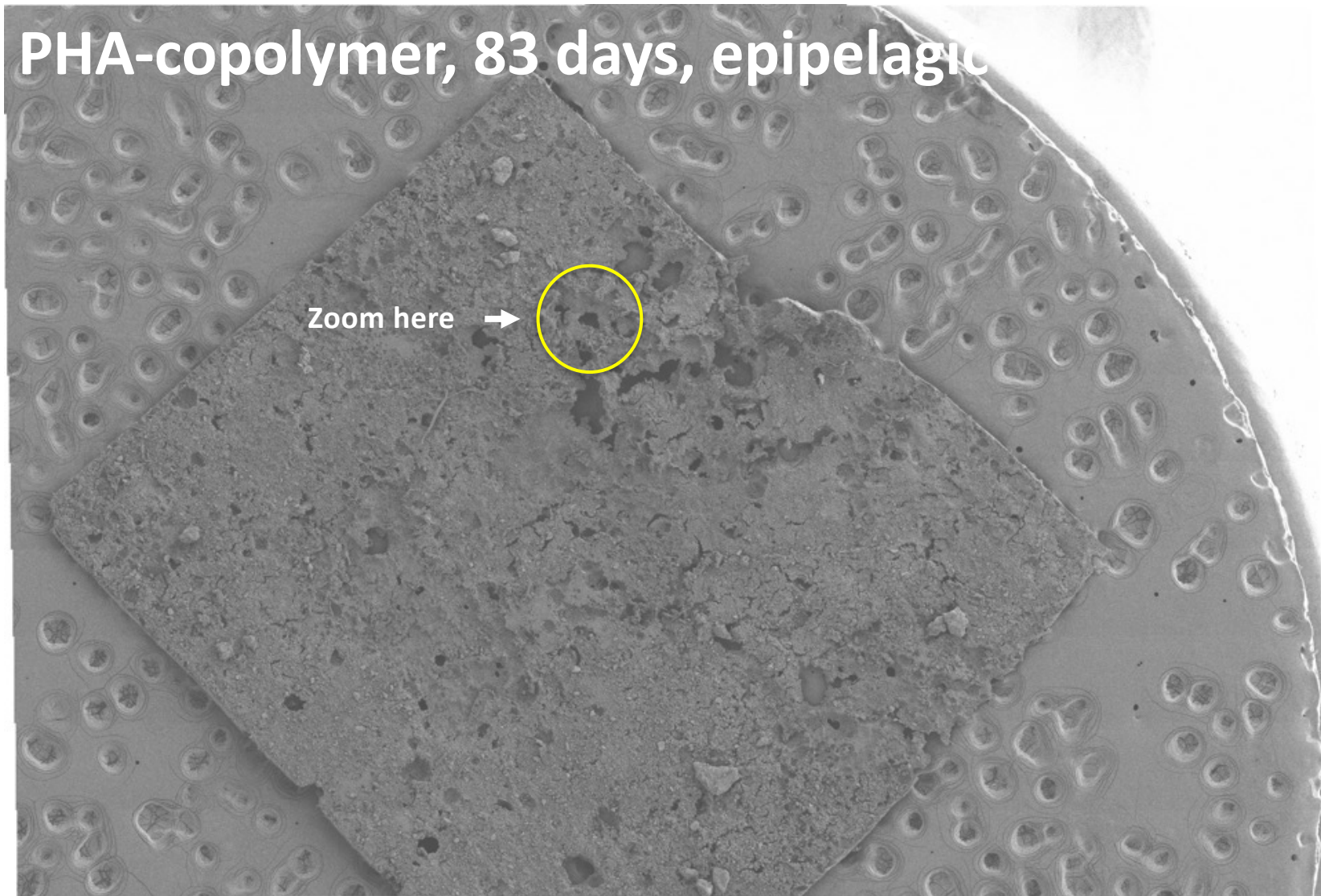


PBSeT: PolyButylene Sebacate co butylenTerephthalate

PHA: Polyhydroxyalkanoate Copolymer

LDPE: Low Density Polyethylene

PHA-copolymer, 83 days, epipelagic



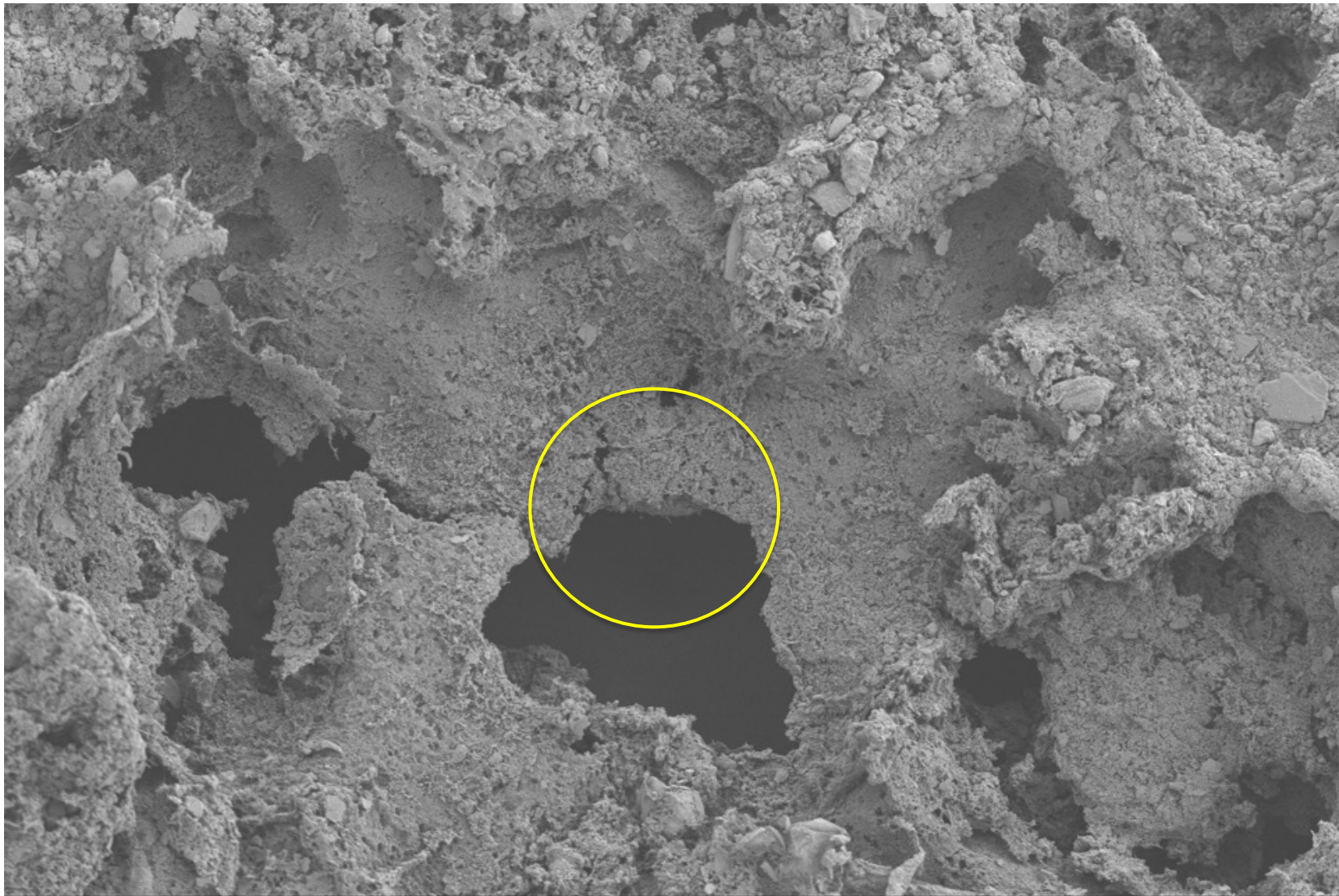


100 μ m
|-----|

EHT = 3.00 kV Signal A = SE2 23 Oct 2019 Mag = 150 X
WD = 9.4 mm Width = 2.008 mm File Name = OB-1B1E-045.tif

MBL

Biological
Discovery
in Woods Hole

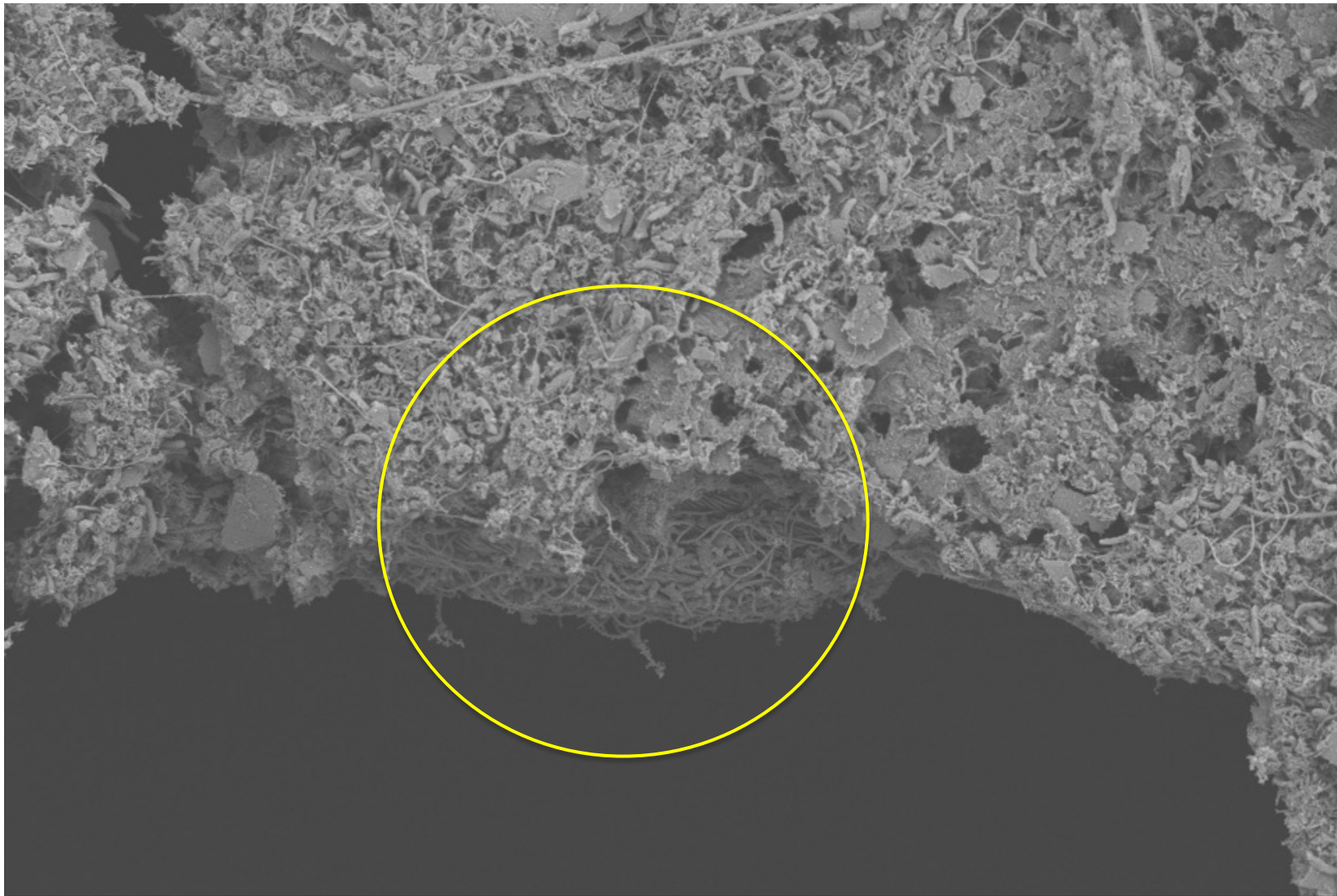


20 μ m
┌───┐
│ │
└───┘

EHT = 3.00 kV Signal A = SE2 23 Oct 2019 Mag = 485 X
WD = 9.4 mm Width = 620.3 μ m File Name = OB-1B1E-044.tif

MBL

Biological
Discovery
in Woods Hole



10 μ m

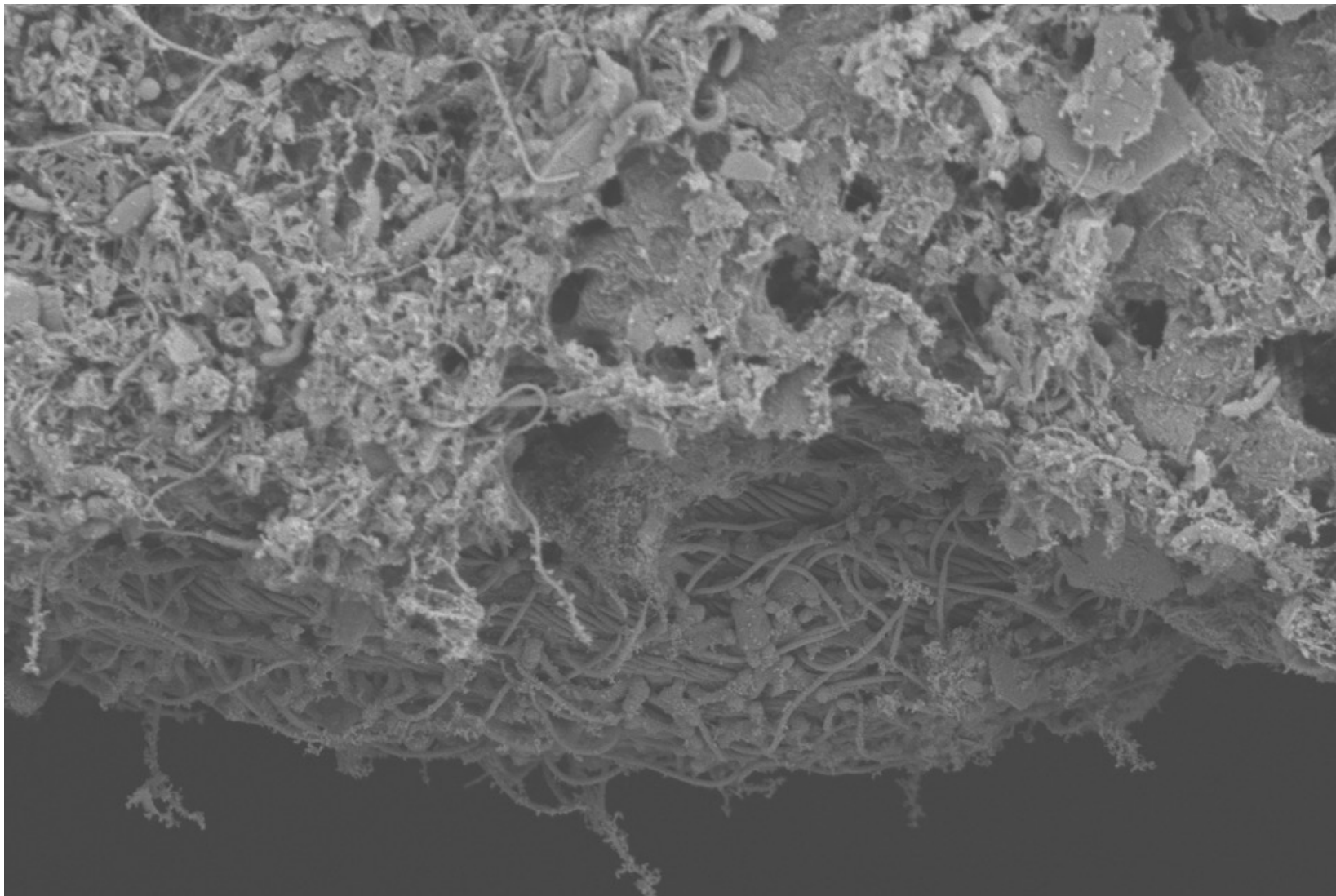


EHT = 3.00 kV Signal A = SE2 23 Oct 2019 Mag = 3.56 K X

WD = 9.4 mm Width = 84.62 μ m File Name = OB-1B1E-046.tif

MBL

Biological
Discovery
in Woods Hole

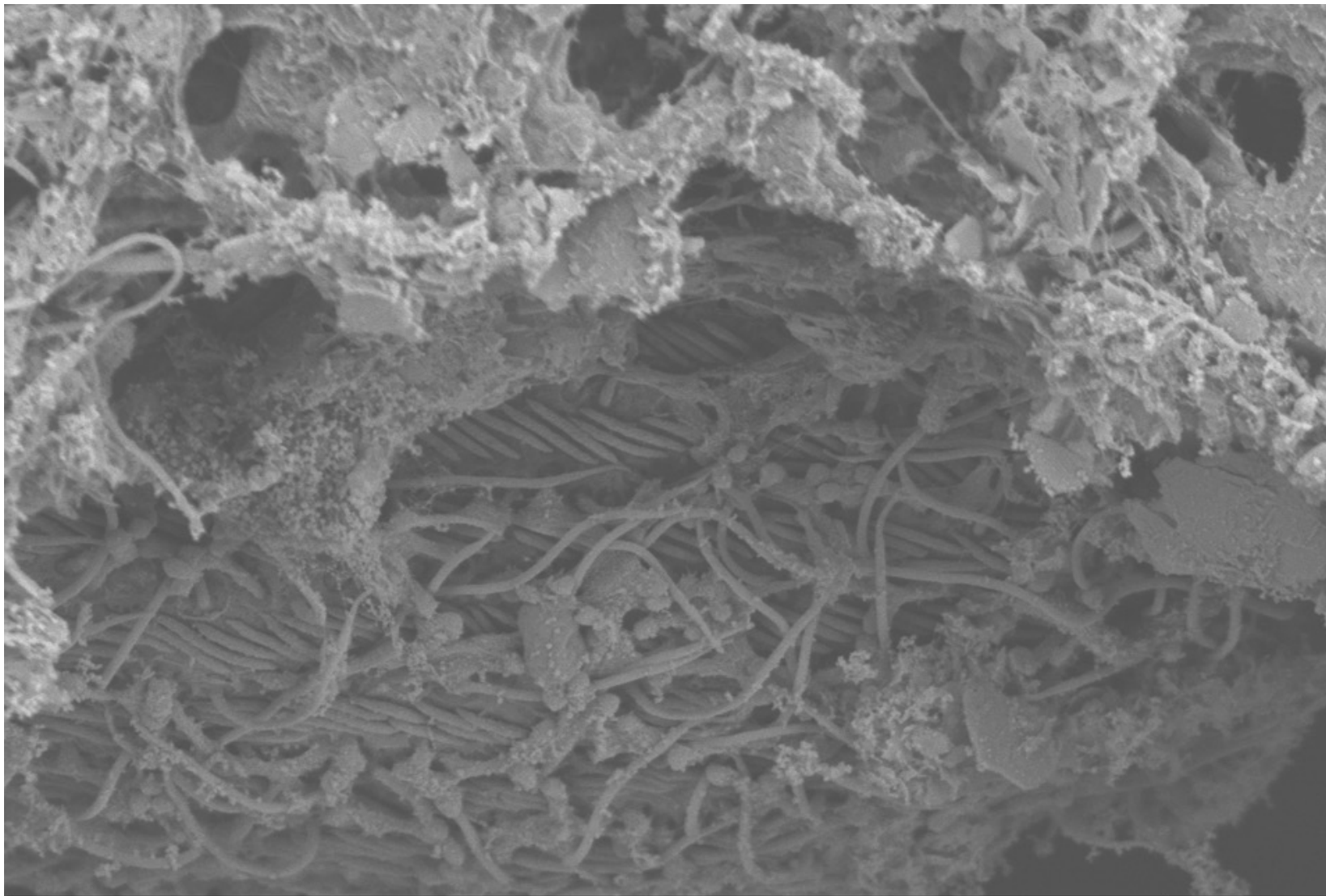


2 μ m
|-----|

EHT = 3.00 kV Signal A = SE2 23 Oct 2019 Mag = 7.92 K X
WD = 9.4 mm Width = 38.02 μ m File Name = OB-1B1E-042.tif

MBL

Biological
Discovery
in Woods Hole

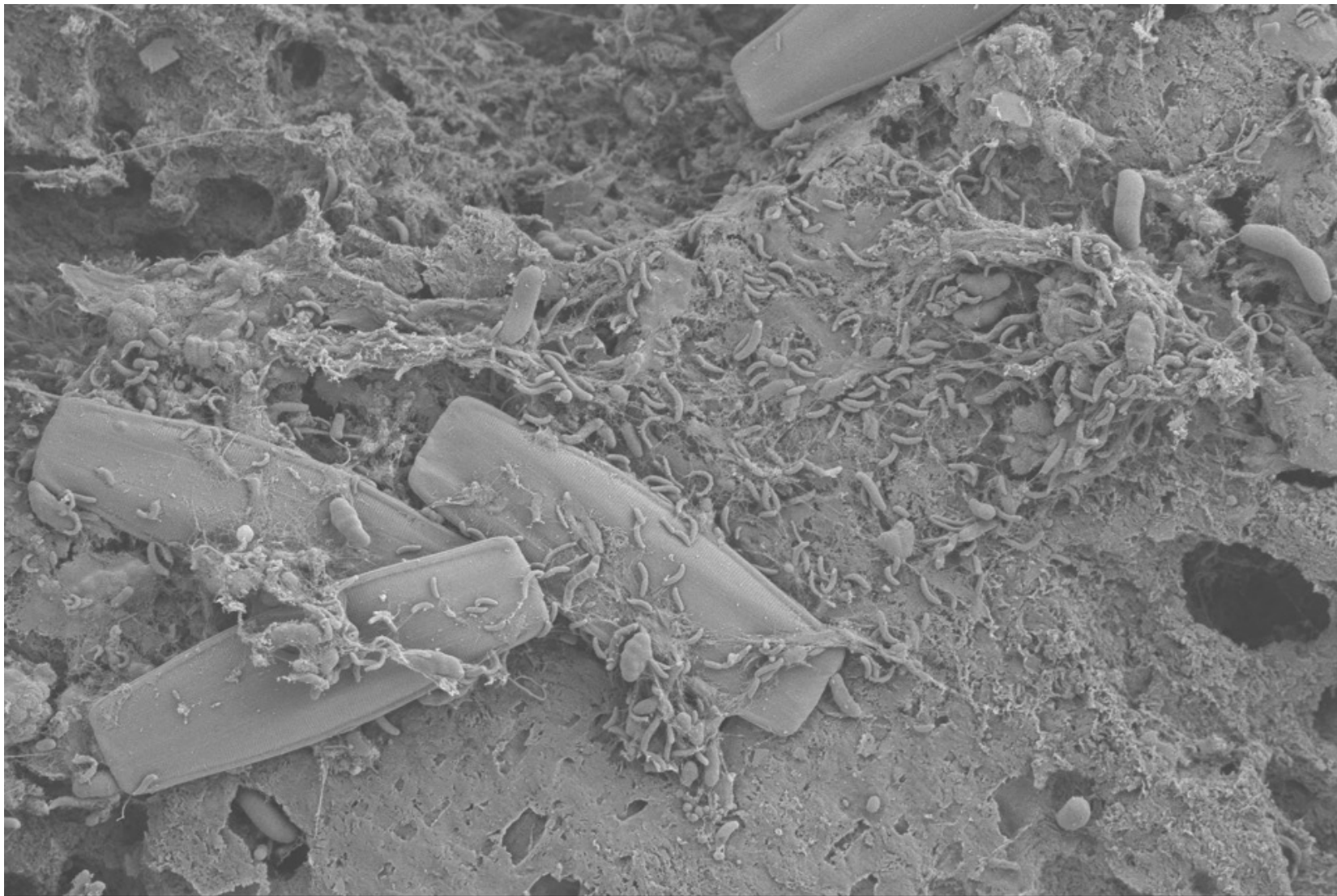


1 μ m
|-----|

EHT = 3.00 kV Signal A = SE2 23 Oct 2019 Mag = 14.37 K X
WD = 9.4 mm Width = 20.95 μ m File Name = OB-1B1E-050.tif

MBL

Biological
Discovery
in Woods Hole



10 μ m



EHT = 3.00 kV Signal A = SE2 23 Oct 2019 Mag = 2.95 K X

WD = 9.3 mm Width = 102.1 μ m File Name = OB-1B1E-025.tif

MBL

Biological
Discovery
in Woods Hole



Outlook

Ongoing Marine Biodegradation Studies with **D6691** original defined consortium:



Article

Comparative Genomics of Marine Bacteria from a Historically Defined Plastic Biodegradation Consortium with the Capacity to Biodegrade Polyhydroxyalkanoates

Fons A. de Vogel^{1,2}, Cathleen Schlundt^{3,†}, Robert E. Stote⁴, Jo Ann Ratto⁴ and Linda A. Amaral-Zettler^{1,3,5,*}

Columbus Instruments Micro-Oxymax

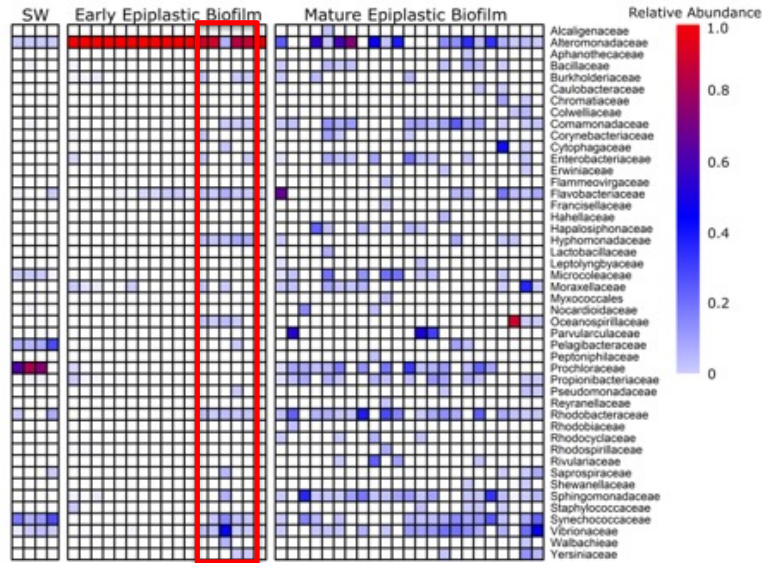


Direct CO₂, O₂ and CH₄ Measurements!

Isolate	Depolymerase Activity	Growth on PHA
<i>Bacillus</i> sp. NTK034	-	-
<i>Bacillus</i> sp. NTK071	-	-
<i>Bacillus</i> sp. NTK074B	✓	✓
<i>Rhodobacter</i> sp. NTK016B	-	-
<i>Vibrio proteolyticus</i> ATCC 15338	✓	N.D.
<i>Vibrio alginolyticus</i> ATCC 33787	-	N.D.

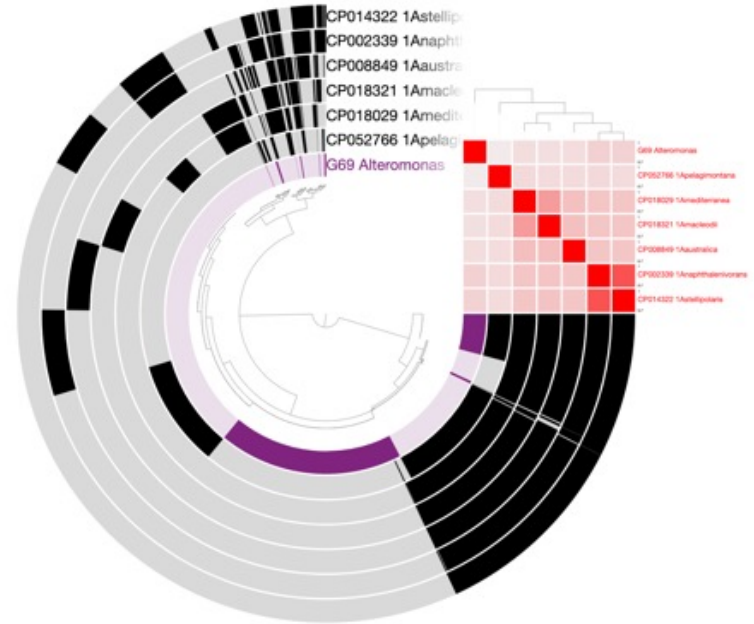
Protein	Oxyanion hole			Lipase box			Catalytic triad			Species											
	Consensus	XXXXX	XXXXX	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX												
1. PHB depolymerase PhaZ7 (Q839Q9)	TEVTPAVI	F	IIH	NGD	GVV	IVV	AV	IV	IV	VSM	GFK	IV	IV	IV	IV	IV	IV	IV	IV	<i>Paucimonas femoignei</i>	
2. Hyp. protein Ssed_3530 (ABV38134)	NEVTPAVI	F	IIH	NGD	GVV	IVV	AV	IV	IV	VSM	GLH	IV	IV	IV	IV	IV	IV	IV	IV	<i>Shewanella sediminis</i> HAW-EB3	
3. Lipase class 2 (ABZ77296)	TEVTPAVI	F	IIH	NGD	GVV	IVV	AV	IV	IV	VSL	GLH	IV	IV	IV	IV	IV	IV	IV	IV	<i>Shewanella halifaxensis</i> HAW-EB4	
4. Hypothetical protein	SEVTPAVI	F	IIH	NS	GVV	IVV	AV	IV	IV	KGI	D	GS	IV	IV	IV	IV	IV	IV	IV	<i>Bacillus</i> sp. NTK074B	
5. Lipase A (P37987)	EHA	GVV	IVV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Bacillus subtilis</i> strain 168
6. Thermostable lipase (Q842J9)	ND	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Geobacillus zailiiae</i> T1
7. Triacylglycerol lipase (P22088)	TEVTPAVI	F	IIH	NGD	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Burkholderia cepacia</i> DSM 3959
8. Triacylglycerol lipase (Q05469)	TEVTPAVI	F	IIH	LAG	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Burkholderia glumae</i> ATCC 6918
9. Triacylglycerol lipase (P26876)	TEVTPAVI	F	IIH	NLG	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Pseudomonas aeruginosa</i> PAO1
10. Triacylglycerol lipase (WP_005101273)	TEVTPAVI	F	IIH	NLIG	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Acinetobacter</i> multispecies
11. Triacylglycerol lipase (WP_064094572)	TEVTPAVI	F	IIH	MAG	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Acinetobacter</i> multispecies
12. Triacylglycerol lipase (WP_005101276)	TEVTPAVI	F	IIH	FGL	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Acinetobacter</i> multispecies
13. Lipase precursor - EC 3.1.1.3	TEVTPAVI	F	IIH	FLG	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Vibrio alginolyticus</i> ATCC 33787
14. Lipase precursor - EC 3.1.1.3	TEVTPAVI	F	IIH	FLG	GVV	IVV	AV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	<i>Vibrio proteolyticus</i> NBRC 13287

Are Early Colonizers also Biodegraders?

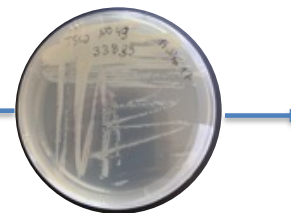
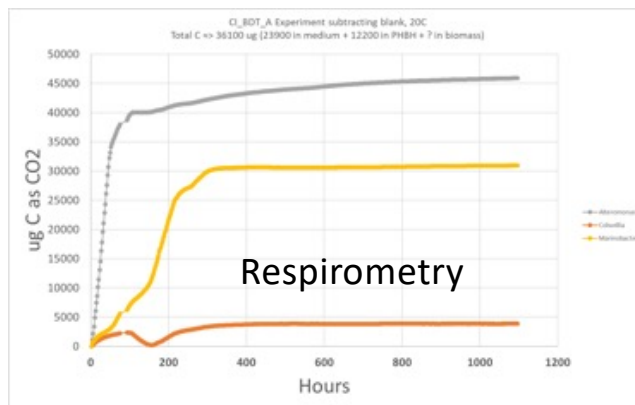


PHA

Bos et al., 2023



Micro-Oxymax
Data

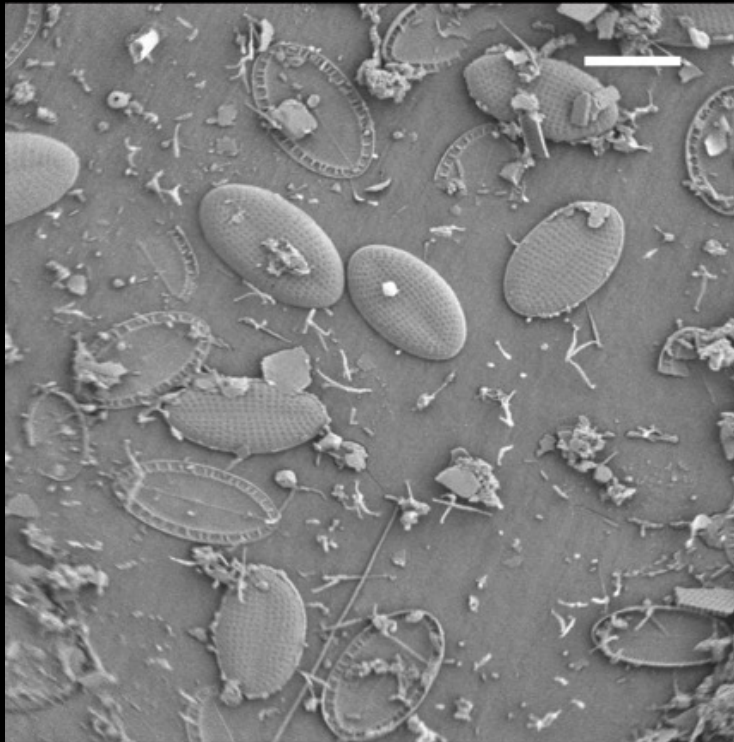


Comparative Genomics

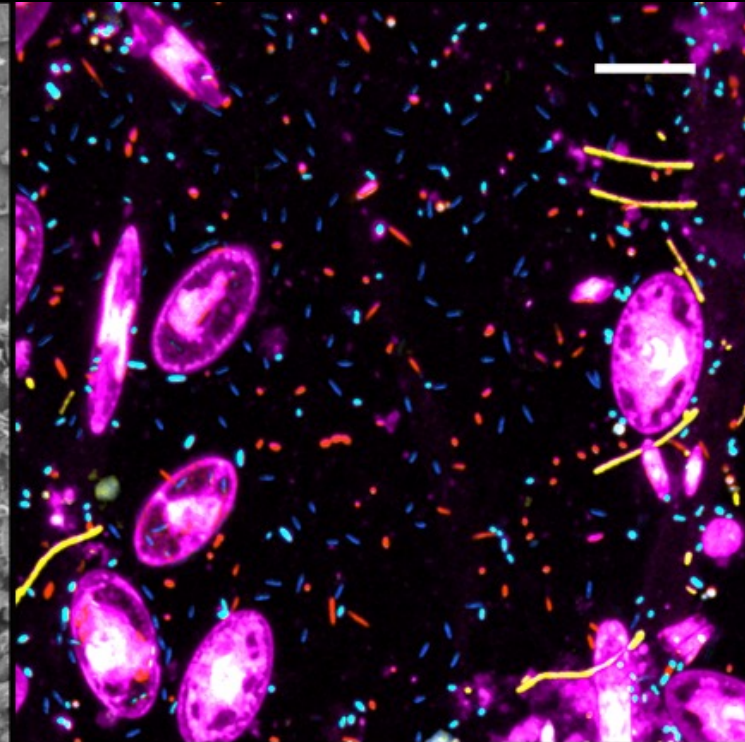


Long read sequencing

Can we open the Biodegradation Black box?



Scanning Electron Microscopy

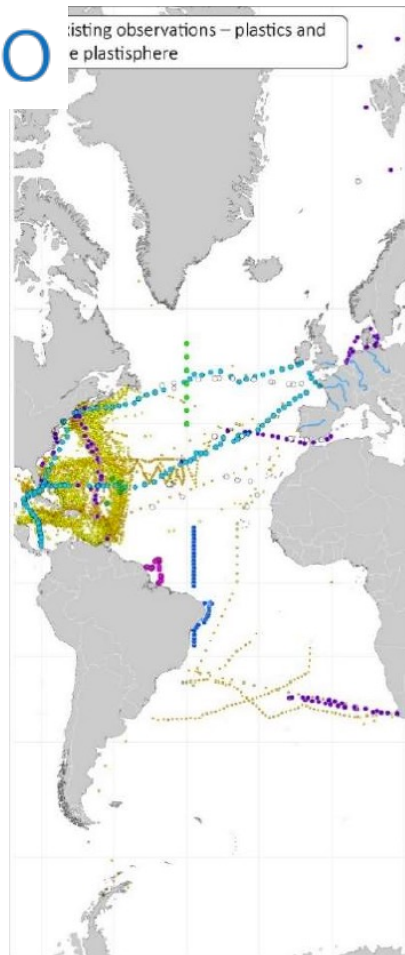


CLASI-FISH
(Combinatorial Labeling and Spectral Imaging
Fluorescence In Situ Hybridization)

Schlundt et al. 2020



Atlantic Ecosystems Assessment, Forecasting and Sustainability



WP2: Plastic and Plastisphere data observations

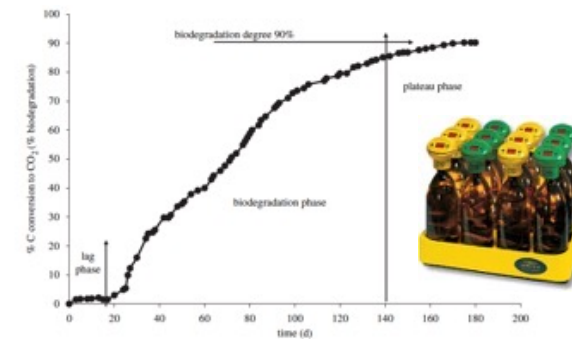
WP5: Network Analysis

Amplicon
Bacteria/Archaea (V4-V5)
Eukaryotes (V4)
Fungi (ITS)

Metagenomes



TransAtlantECO Biodegradation Test*



Song et al., 2009
doi:10.1098/rstb.2008.0289

Test material	No. of replicates
PHBH	3
PLA	3
LDPE	3
OXO-PE	3
Cellulose filter (+ control RRT)	3
Blank	3
Total	18

*Proposed project requires funding

Take-home Messages on Plastic Debris and Biodegradation

- 1 There is one Ocean shared by all: litter doesn't respect EEZ's
- 2 Marine environments are not homogeneous
- 3 Out of sight shouldn't be out of mind when it comes to biodegradation
- 4 **Reduce**, Reuse, Recycle
- 5 Biodegradable plastic is a **part** of the solution to the plastic problem
- 6 Proper waste infrastructure and composting facilities are required
- 7 **Global problems require global cooperation – collaborative efforts across International standardization bodies**

It's time to start caring about what's on the bottom of the ocean

The New Yorker



Barnes et al., 2009.



Saxon, (c) 1983 The New Yorker

"I don't know why I don't care about the bottom of the ocean, but I don't."

THE PLASTISPHERE 1945.36

Thanks for your attention!
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[@plastisphere](https://twitter.com/plastisphere)

MBL Central Microscopy Facility (Woods Hole, USA)
Gulbenkian Sequencing Facility (Oeiras, Portugal)
University Medical Center (Amsterdam, NL)
MMB department (NIOZ)
Leslie Murphy, Fons de Vogel, Lia Corbett, Ryan Bos, Chris Dupont, Tracy Mincer, Erik Zettler

An Artist's Interpretation of the "Plastisphere" - Pinar Yoldas

