Biodegradation Studies and Experiments for Materials in the Marine Environment Series

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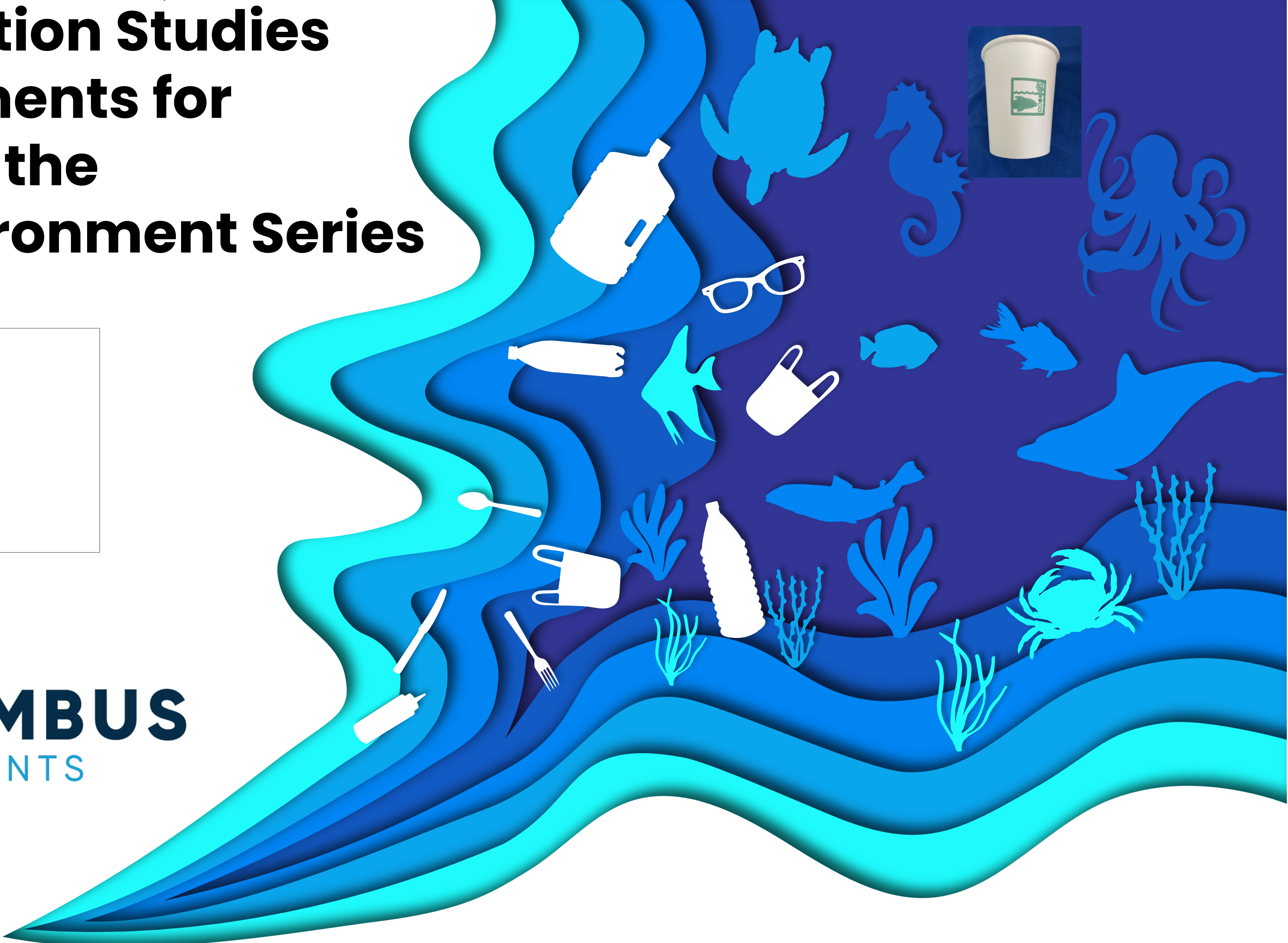


Biodegradation Studies and Experiments for Materials in the Marine Environment Series

Part 1: A Tiered Approach for Studying Polymers in the Marine Environment: Criteria, Best Practices and Test Methods

June 21st, 2023 | 11aEST 5pCET

Speakers:

Jo Ann Ratto, D. Eng.
Adjunct Faculty

University of MA Lowell, Plastics Engineering

UMASS LOWELL

Chris Thellen, Ph.D.
Product Development Engineer
Endurans™ Solar (Worthen Industries).

endurans solar

Biodegradation Studies and Experiments for Materials in the Marine Environment Series

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

July 19th, 2023 | 11aEST 5pCET

Speakers:

Linda Amaral-Zettler, Ph.D.
Research Leader
NIOZ Royal Netherlands Institute for Sea Research

Biodegradation Studies and Experiments for Materials in the Marine Environment Series

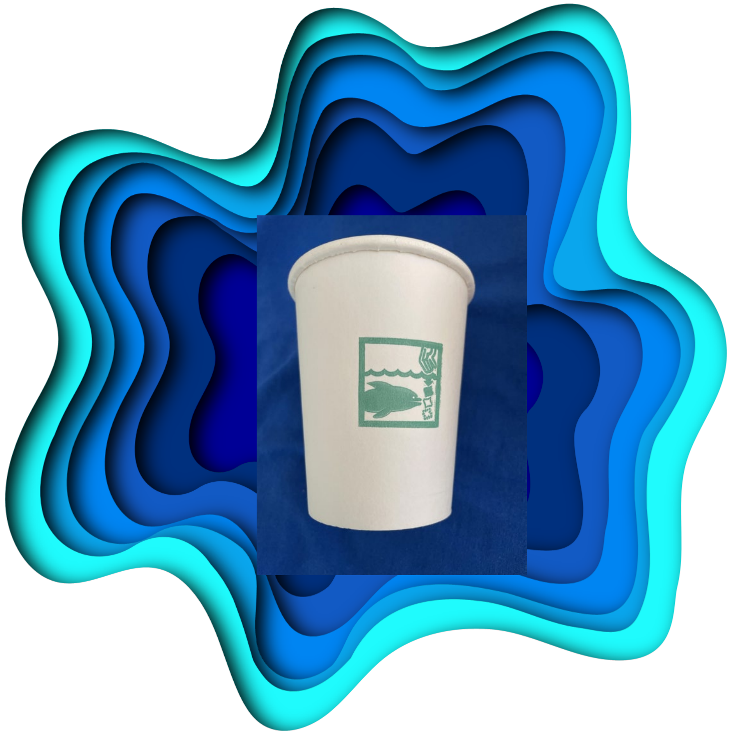
Part 3: New State of the Art Laboratory / Facility for Investigation of Materials in the Marine Environment

August 16th, 2023 | 11aEST 5pCET

Speakers:

Micheline Labrie, Ph.D.
Science Lead & Manager/Co-principal Investigator
University of Massachusetts Dartmouth (UMassD)

UMass Dartmouth



Part 1: A Tiered Approach for Studying Polymers in the Marine Environment: Criteria, Best Practices and Test Methods

Outline

Marine Debris – Global Problem

- Global plastics waste examples

Biodegradable Polymers

- What are they?
- Historical Work Targeting Biodegradables for the Ocean
- Polymers that degrade in the marine environment
- Is there value to use for waste reduction?
    - Possible solution to marine debris?

Testing for Biodegradable Polymers

- Tier 1, Tier 2, Tier 3 Methods for Biodegradation
- Disintegration
- Toxicity

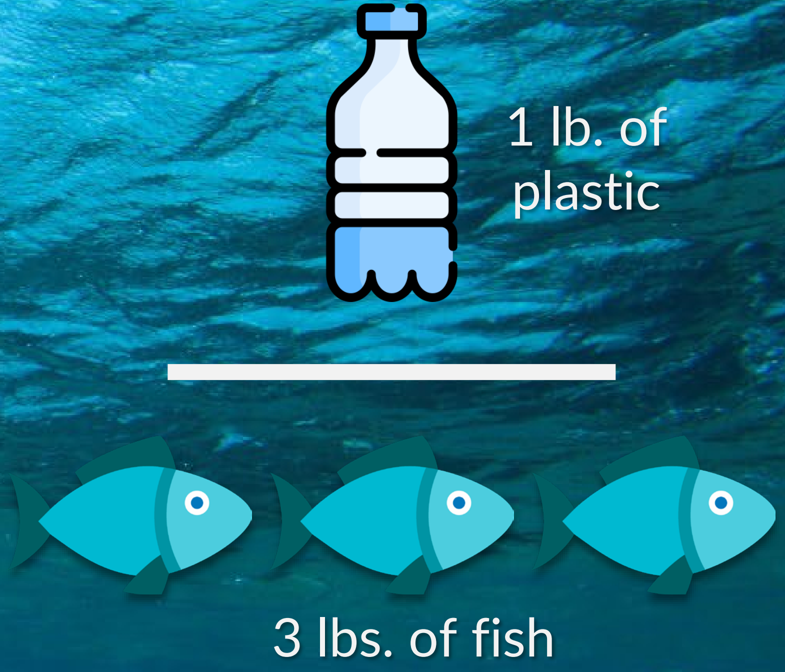
Questions for Panel

Marine Debris
A Global Problem

By 2025, 250 million tons of plastics in the oceans

Source: https://www.surfrider.org/coastal-blog/entry/its-official-ca-gov.-brown-signs-bag-ban-bill

By 2025, 250 million tons of plastics in the oceans



Source: https://www.surfrider.org/coastal-blog/entry/its-official-ca-gov.-brown-signs-bag-ban-bill


Yamuna River in New Delhi

Photo: Manan Vastsyayana


Bulgarian Reserve

Henderson Island

Henderson Island

Sources: www.pnas.org/cgi/doi/10.1073/pnas.1619818114

Henderson Island

Crabs make homes in plastic containers

Green turtle entangled in fishing lines

Plastic items accumulated along the tide line

Sources: www.pnas.org/cgi/doi/10.1073/pnas.1619818114

Artic Ocean

Wildlife Everywhere

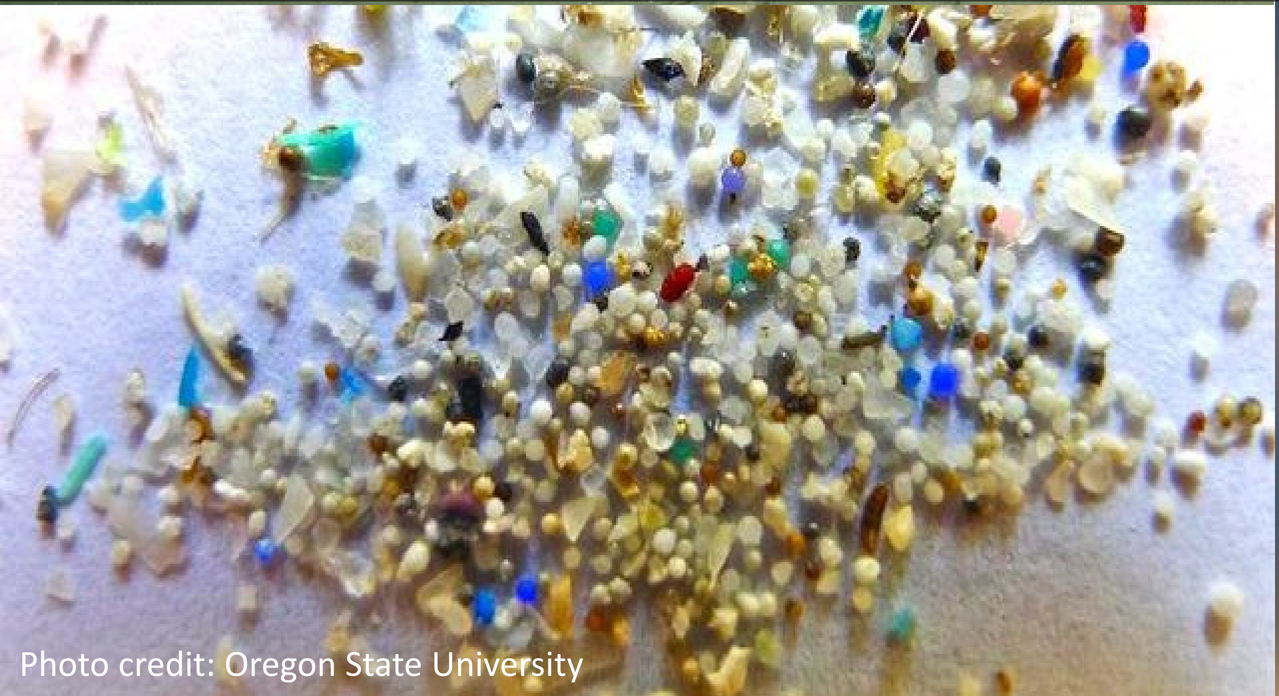


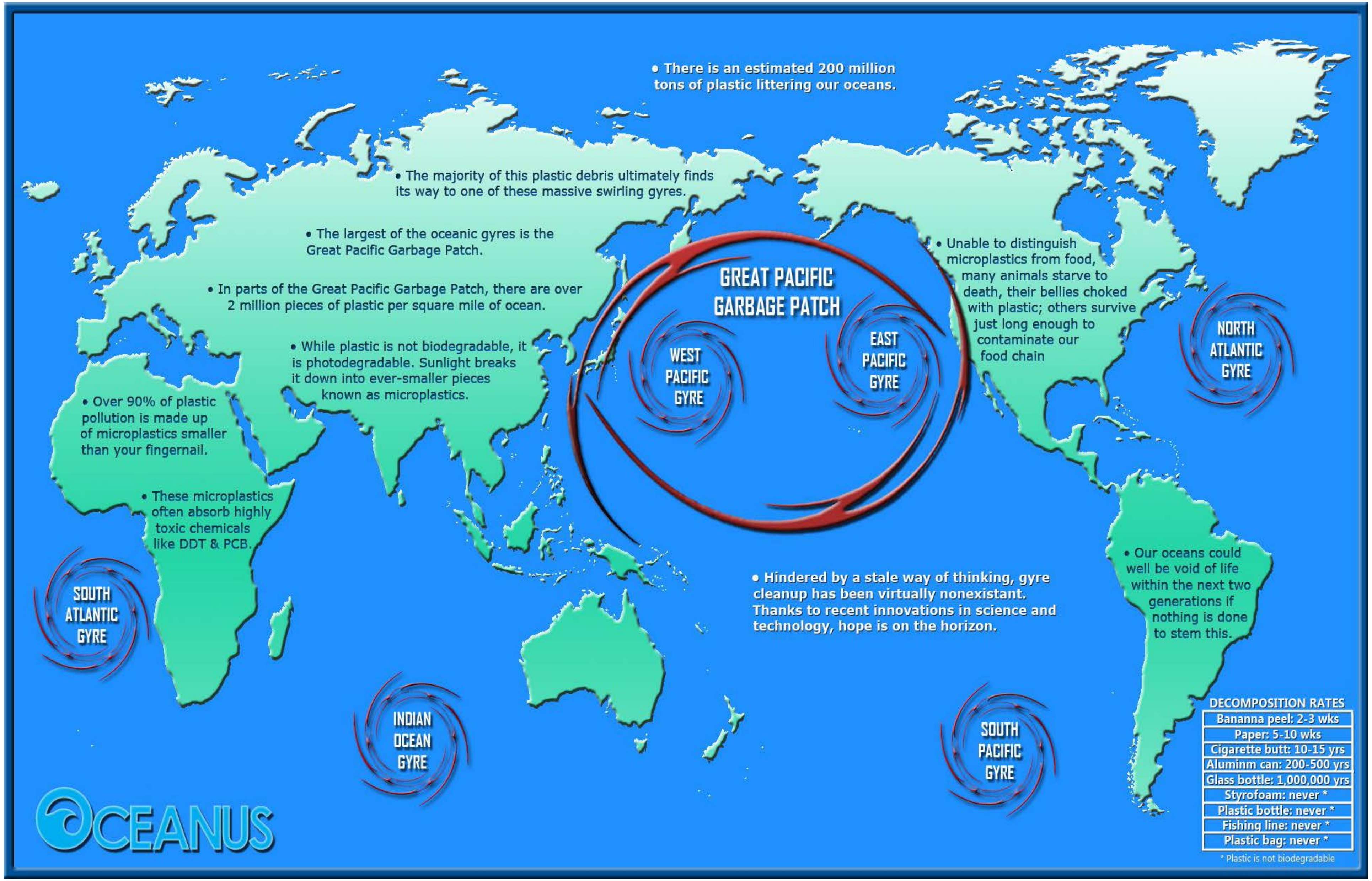
Photo credit: Oregon State University

Photo Source: Greenhouse Carbon Neutral; Nature, 2013

"DEAD WHALE, 220 POUNDS OF DEBRIS INSIDE, IS A 'GRIM REMINDER' OF OCEAN TRASH"

- ***"The sperm whale washed up on a Scottish beach with a stomach full of rope, netting and plastic. "What was unusual in this case was the sheer volume," a local expert said."*** **----- The New York Times.**

Garbage Patches in the Oceans



Source: Science, 2015

By 2050, our Earth will have 33 billion tons of plastics debris

Source: http://www.geologyin.com/2016/02/plastic-has-become-part-of-earths.html#more

Biodegradable Polymers

What are they? Definition:

- **Biodegradable Polymer:** A material which can be metabolized by microbes as a food source resulting in carbon dioxide, water and new cell material as end products.

- **Biodegradability**: Assessed by determining the proportion of polymer-C converted to biogas-C. The percent of theoretical gas production, expressed as a fraction of the measured or theoretical carbon content of the test material, is reported as a function of time.

Historical Work Targeting Biodegradables for the Ocean

Goal: Develop novel plastics that seek to reduce the burden of waste generated at sea.

Biodegradation Studies in cooperation with:
Woods Hole Oceanographic Institution, US Navy, US Army, Universities and Industry

Certified Biodegradation Laboratory

Army obtained (December 2012) an ISO 17025 audit that led to certification from UL Environmental Inc. for testing of biodegradation, toxicity and disintegration of polymers in the marine environment in accordance with American Society for Testing and Materials (ASTM) (ASTM D7081, Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment)
Army was also certified by the Biodegradable Products Institute (BPI) for these methods/specification. (2007-2012)

Interlaboratory Study with ASTM Method

"Test Method For Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations" Four Laboratories participated in an Interlaboratory study using the same positive and negative controls and biodegradable polymers to determine rate of weight loss as function of time in different ocean environments (Locations: MA, CA, RI, FL)

Historical Work Targeting Biodegradables for the Ocean

Applications:

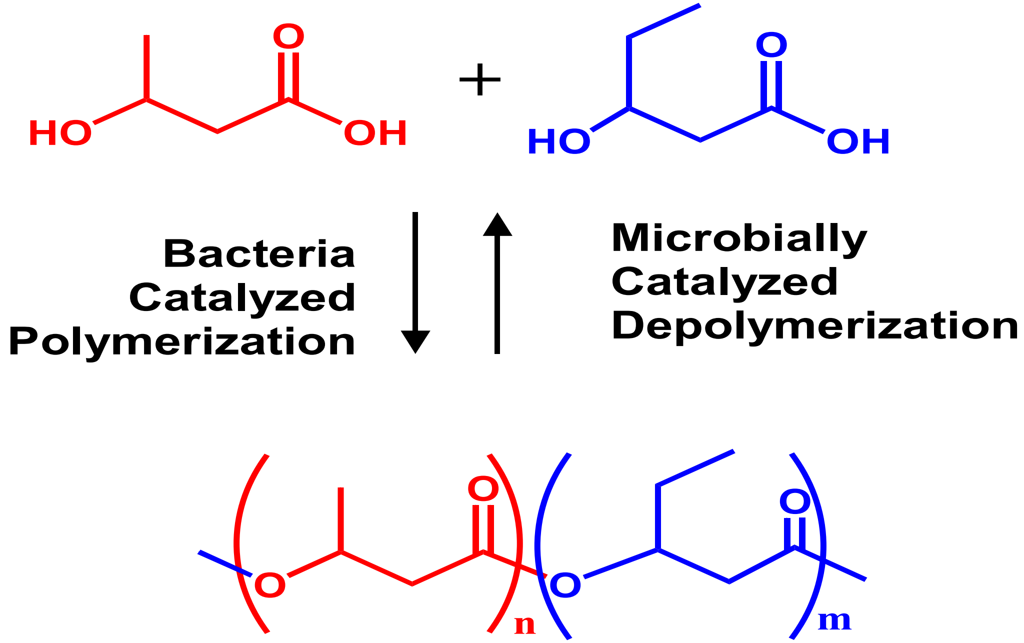
- Straws
- Trash bags
- Blow Molded Bottles
- Utensils
- Dinner Ware – plates, bowls, trays
- Coated Paper
- Cups – Coated Cups
- Variety of materials for collaborative research projects

Polymers that Degrade in the Marine Environment ???

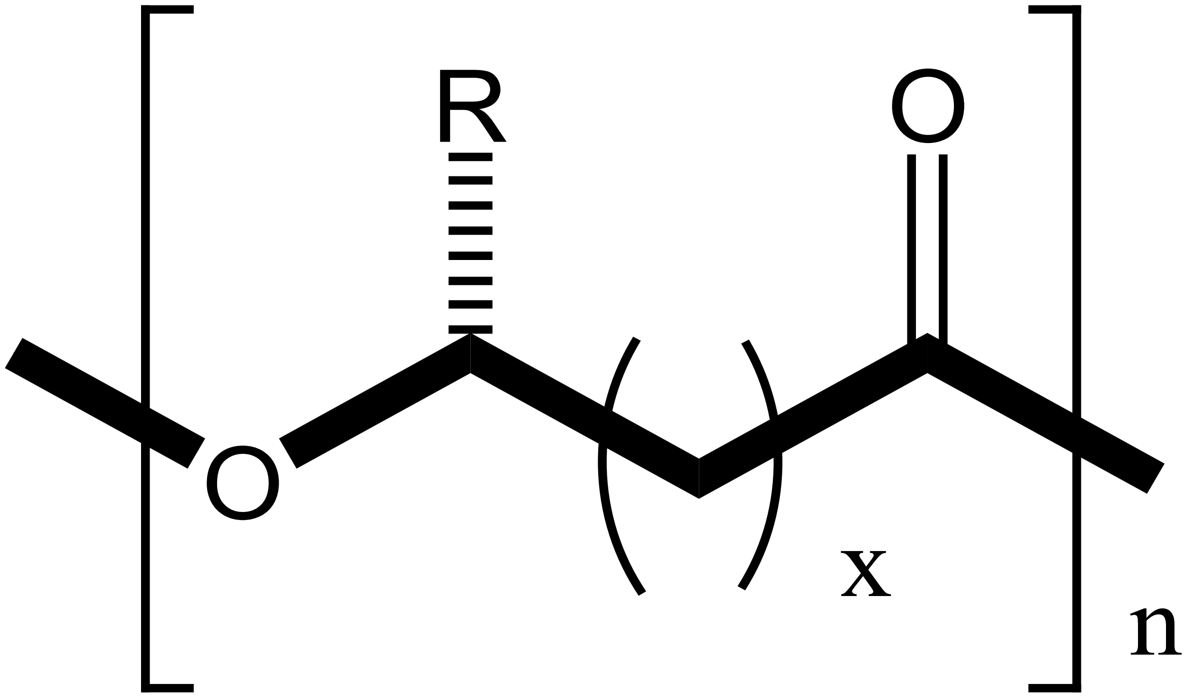
- Polyhyroxyalkanoate
- Polycaprolactone
- Polysaccharides
  - Starch
  - Soy Protein
  - Cellulose
  - Levan
  - Pullulan
  - Chitosan / Chitan
- Kraft Paper
- Wheat Gluten

Biodegradable Polymers in Marine Environment

Polyhydroxy buterate valerate (PHBV)



PHA General Structure



versatile range of structures: homopolymers, copolymers, terpolymers, block-copolymers

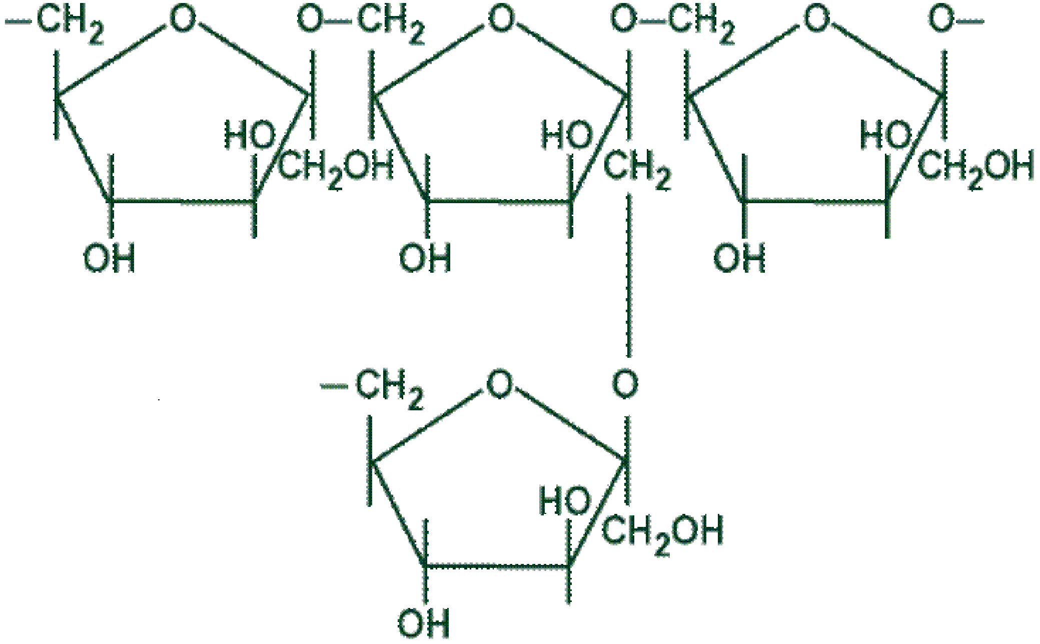
Polyhydroxyalkanoate

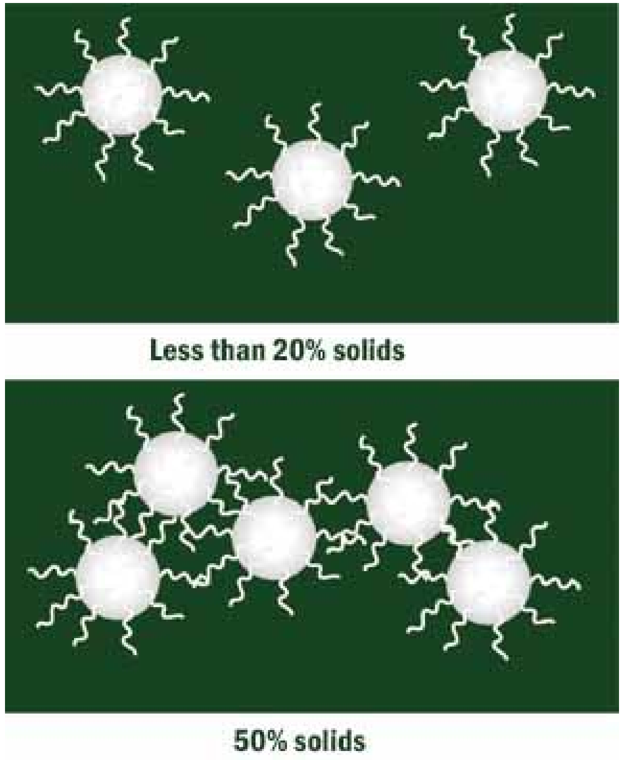
- Biobased aliphatic polyester
- Semi-crystalline thermoplastic
- From flexible to rigid, properties vary from near LDPE to HDPE, PP, PS
- Withstands hot liquids, HDT >120°C
- (e.g. coffee, soup)
- Chemical resistance similar to PET
- Good barrier and grease resistance
- Good printability

Polyhydroxyalkanoate

- Suitable grades for cast and blown film applications
- Can be processed on conventional equipment and infrastructure
- Excellent melt strength
- good drawdown stability for blown film processing
- Heat sealable
- Excellent tensile properties
- suitable puncture toughness and tear resistance
- Contact clarity

Structure of Levan Polysaccharide





Mw > 10 million Daltons

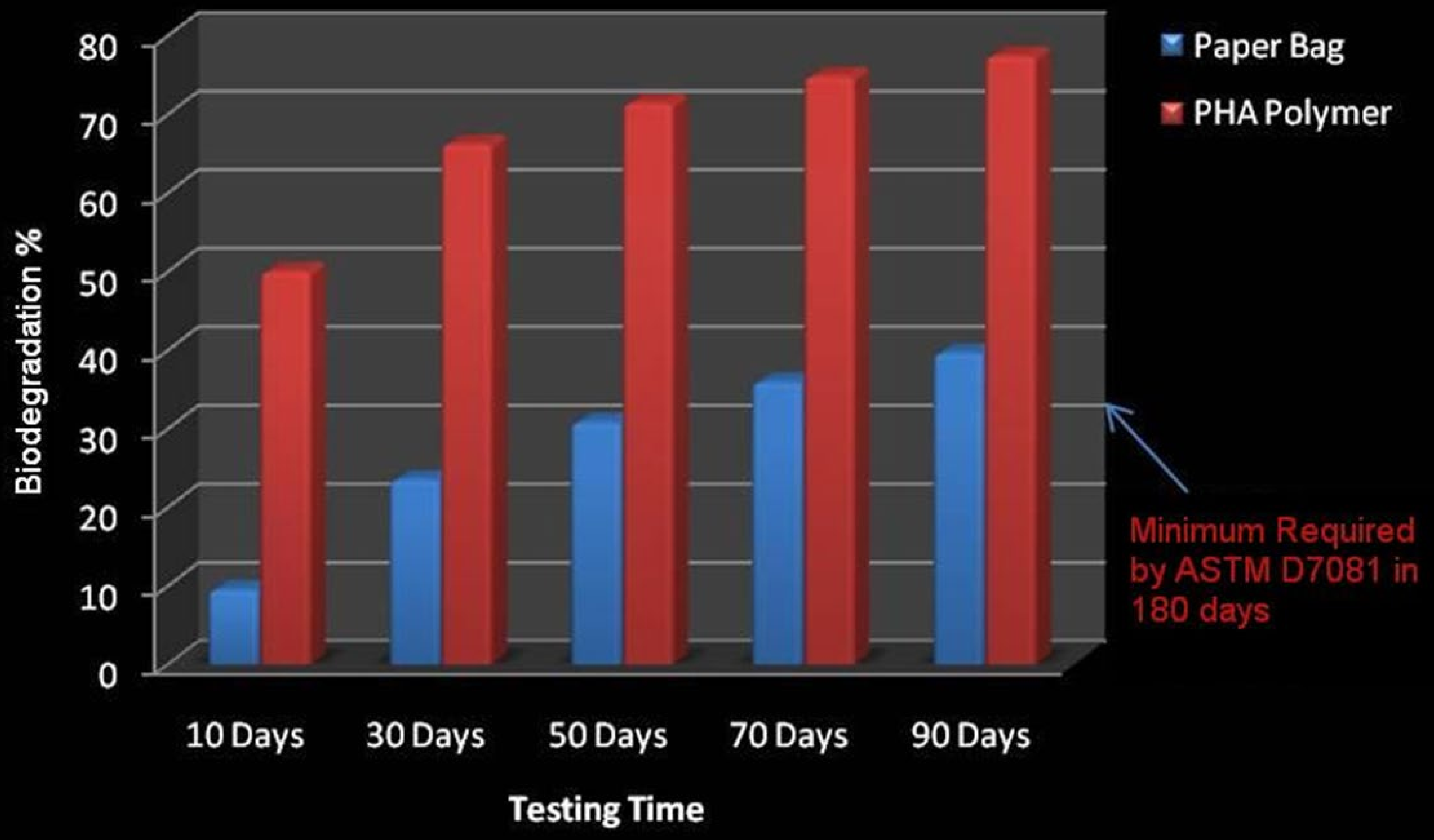
*

- Solutions containing less than 20% solids exhibit newtonian behavior in water.
- At 50% solids, the polysaccharide resembles chewing gum

* Figure taken from www.polysaccharides.us

Is there value for waste reduction?

Biodegradation Testing of PHA Polymer vs. Paper Bag



Testing for Biodegradable Polymers

Tier Testing Approach

I

- Screening - Standard methods in laboratory
  - Respirometry methods
  - ASTM 6691
    - Standard Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum

II

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

III

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

Tier 1

ASTM 7081

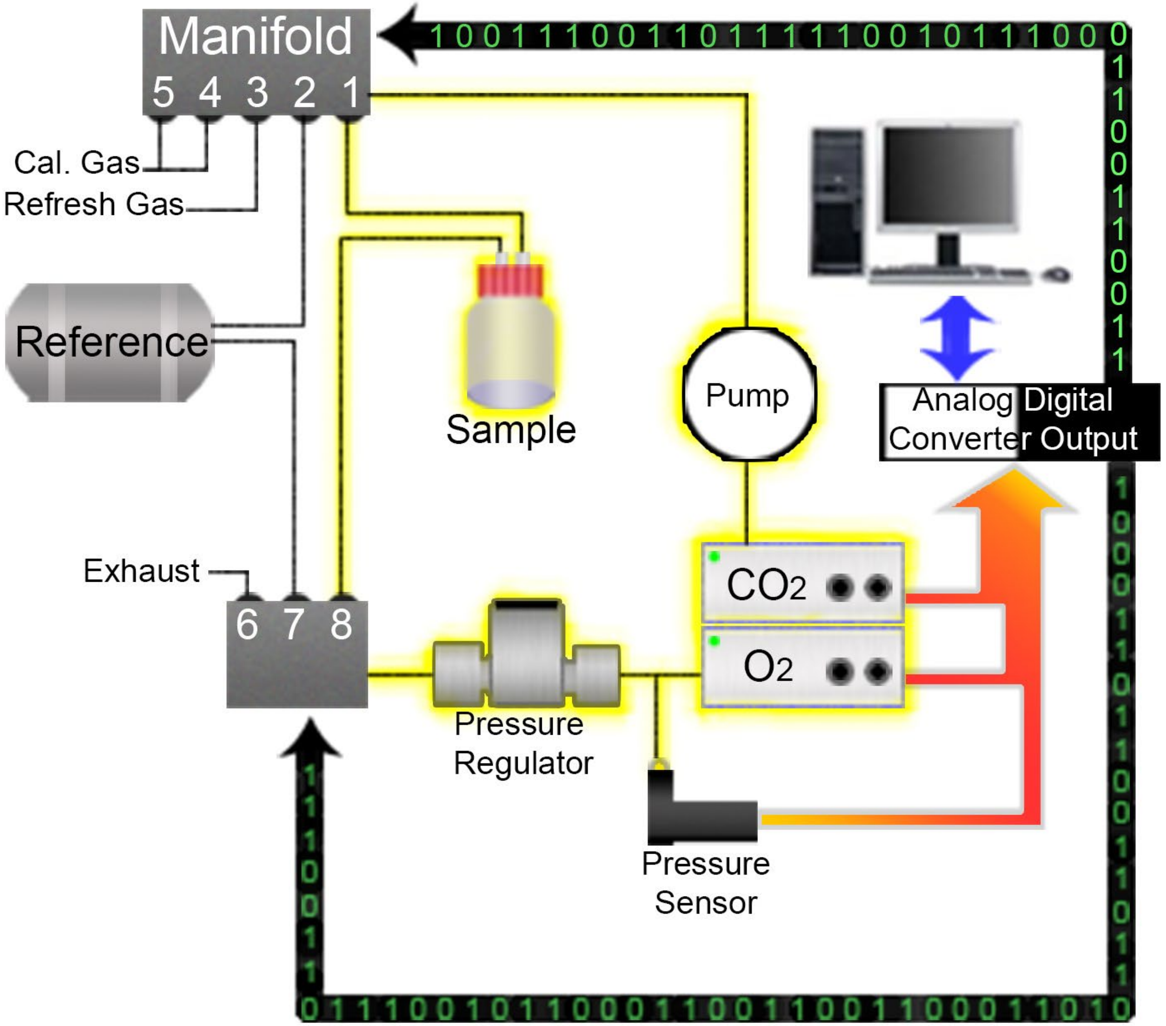
Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment

- Columbus Instruments Micro-Oxymax system
- 80 vessels
- NDIR Carbon dioxide and methane sensors
- Capability to do ASTM aerobic and anaerobic testing for soil, compost or marine environment.
- Includes software for experiment setup, data collection, and real-time gas production.


Sample Drier
Micro-Oxymax System Sample Pump
Condensing Air Drier
Power Supply
ORS 200
BOEKEL Grant
COLUMBUS
INSTRUMENTS


COLUMBUS
INSTRUMENTS

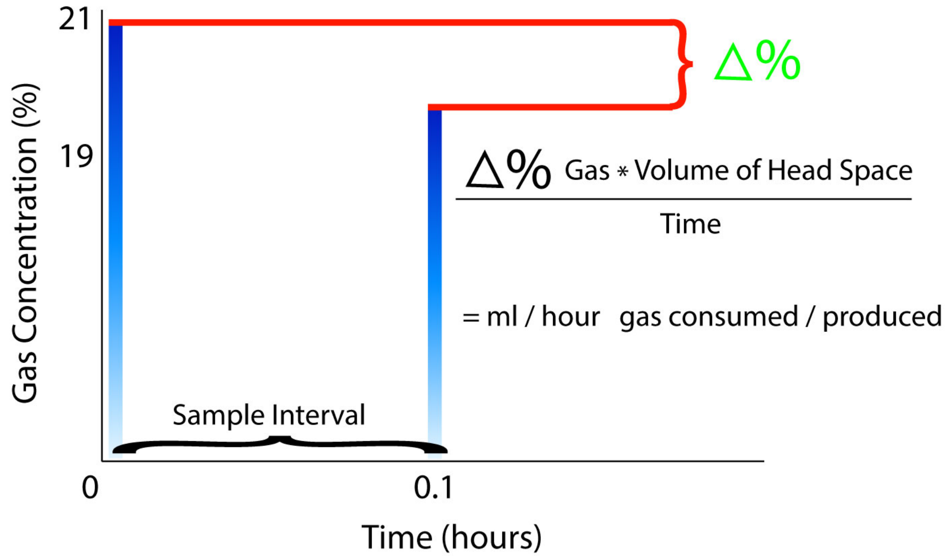
Micro-Oxymax Closed-Loop Measurement Method



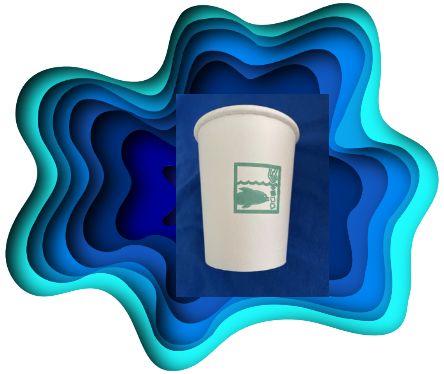
$$V_T = \frac{V_R + V_S}{\left(\frac{(P_4 - P_A)}{(P_5 - P_A)}\right) - 1}$$

...where:

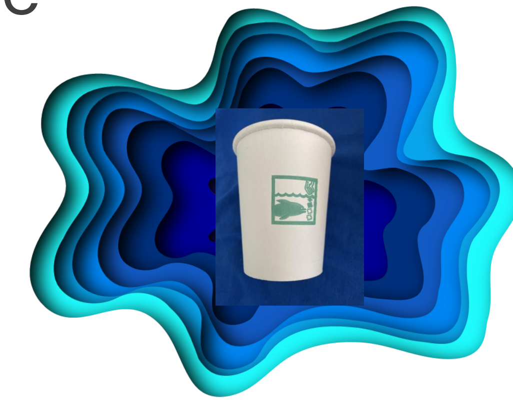
V_T = Sample Chamber Volume
V_S = Sensor Volume
V_R = Reference Chamber Volume
P_A = Barometric Pressure
1 = a remainder from the cancellation of units



ASTM Methods and Specification



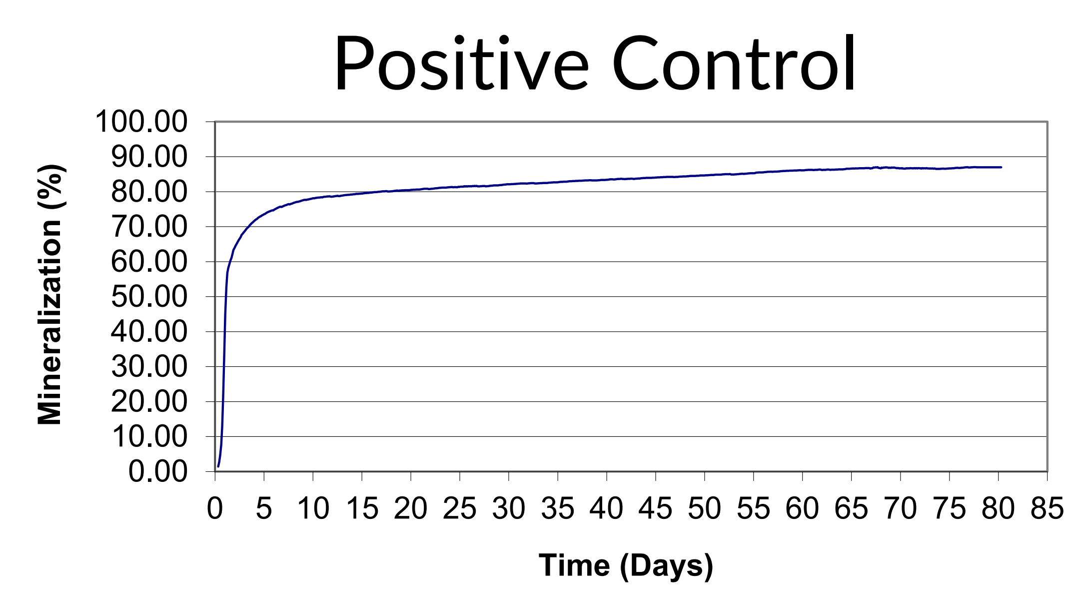
- D6691, Title: Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment
- D7081 Title: Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment
- ASTM D7473 Test Method for Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations



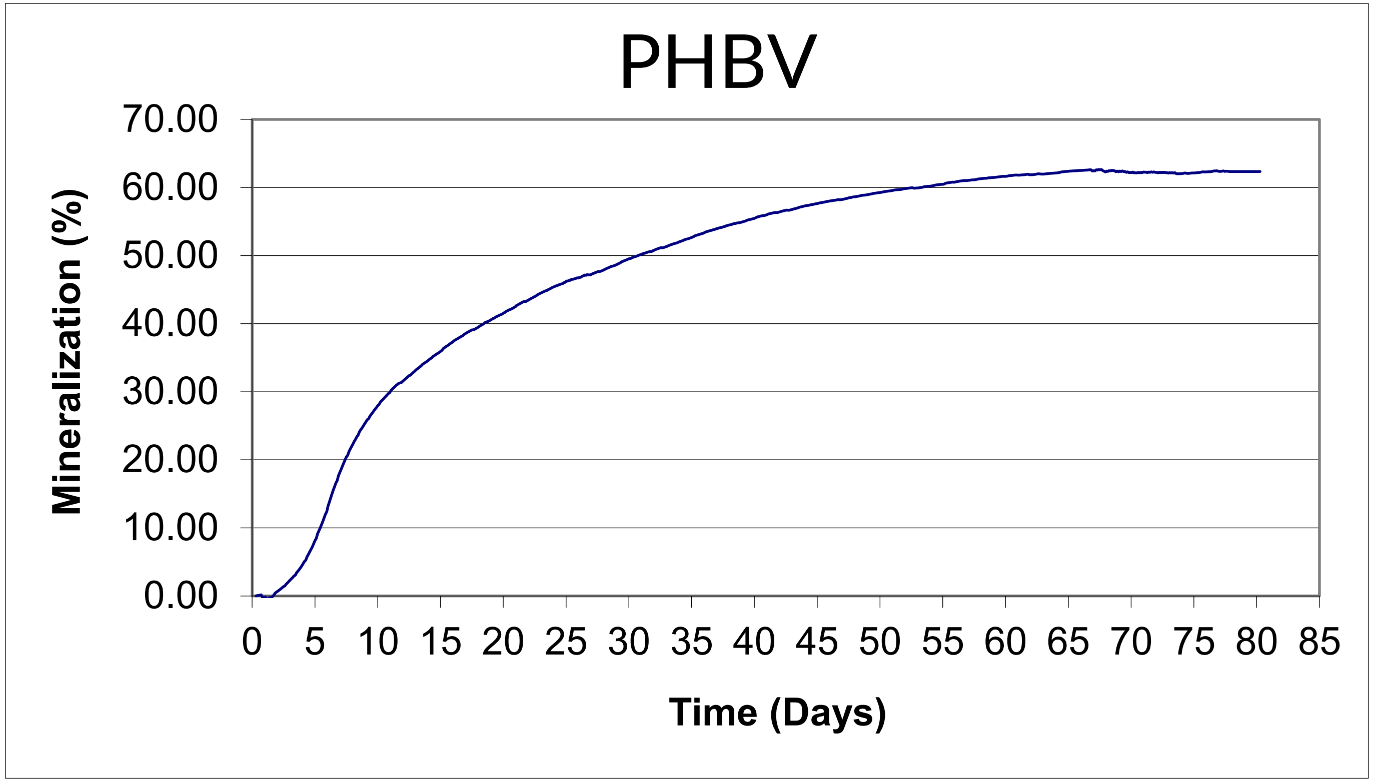
This Tier 1 test method consists of the following:

- Performing a screening to see if a material is biodegradable in the marine environment.
- Optimizing conditions to promote biodegradation (i.e. temperature for organism, increased surface area of the material (cryogenically milling the sample to certain particle size)
- Determine the carbon content of sample for % mineralization
- Preparing a uniform inoculum of various isolated marine microorganisms or using natural sea water with nutrients.
- Exposing the test materials to the inoculum / sea water
- Using a respirometer to measure the total biogas (CO_2) produced as a function of time
- Assessing the degree of biodegradability.

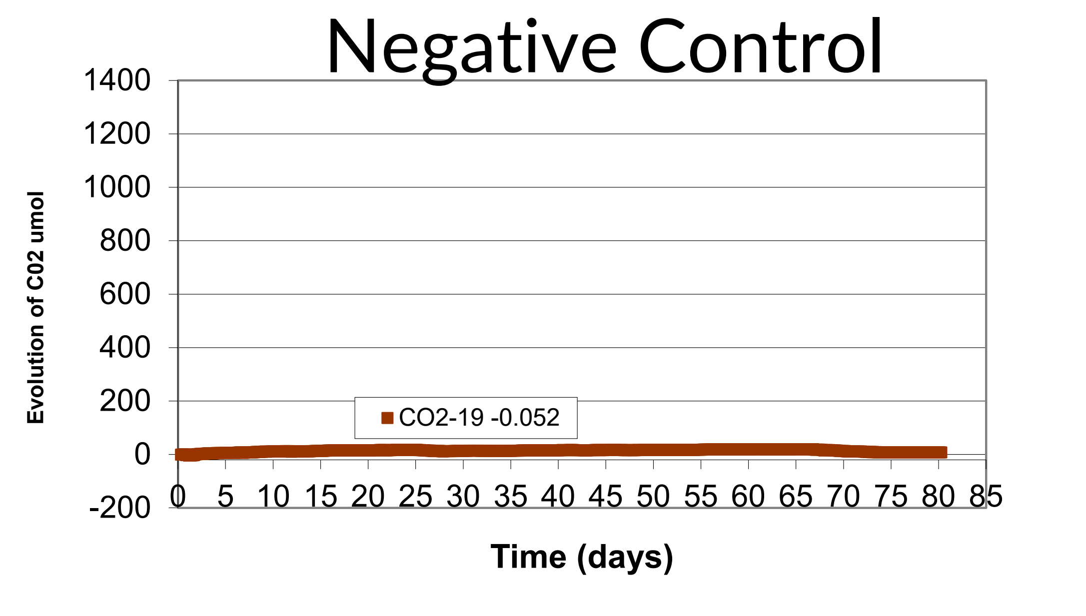
Tier I PHBV



Positive Control
Mineralization (%)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)

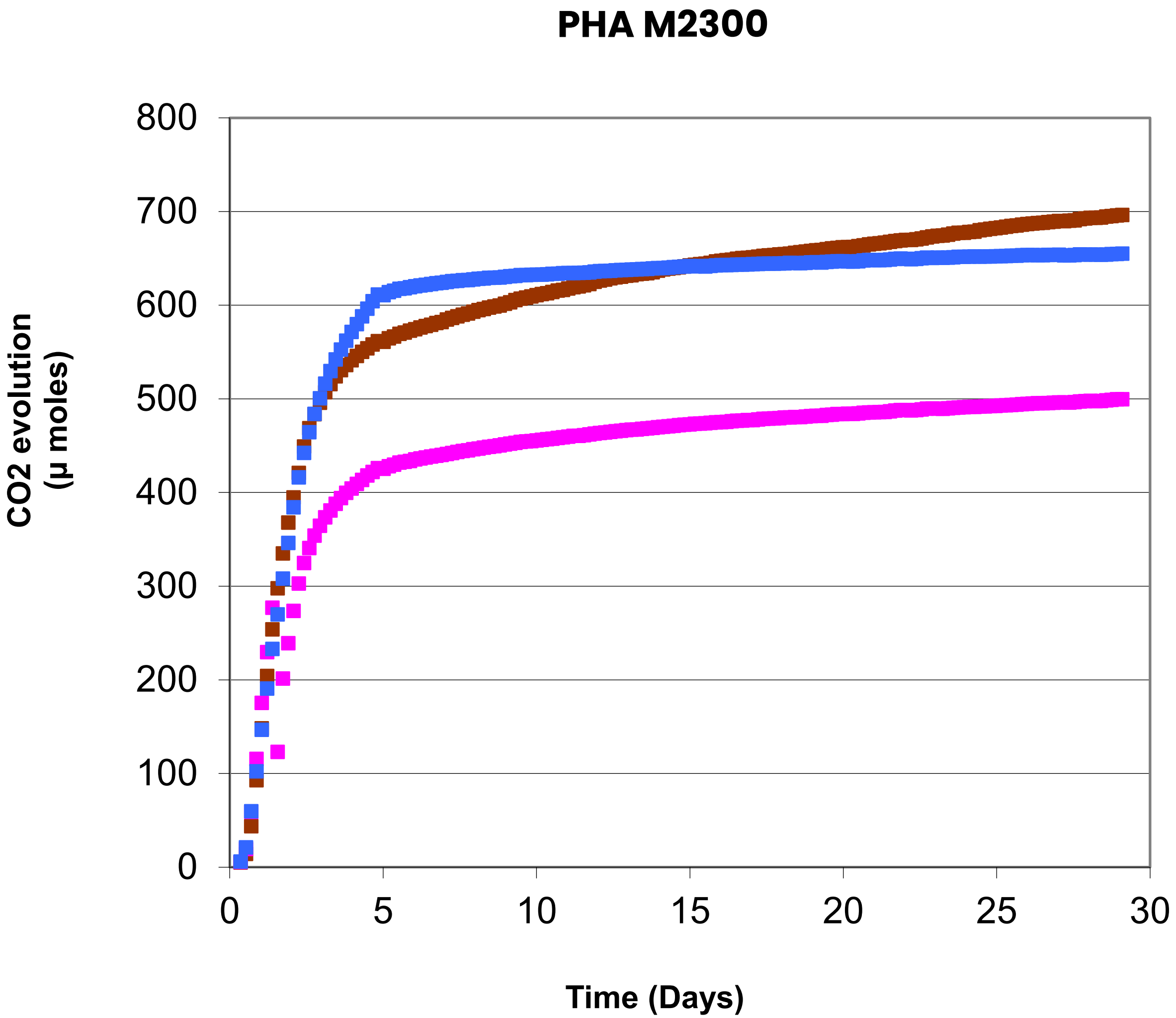


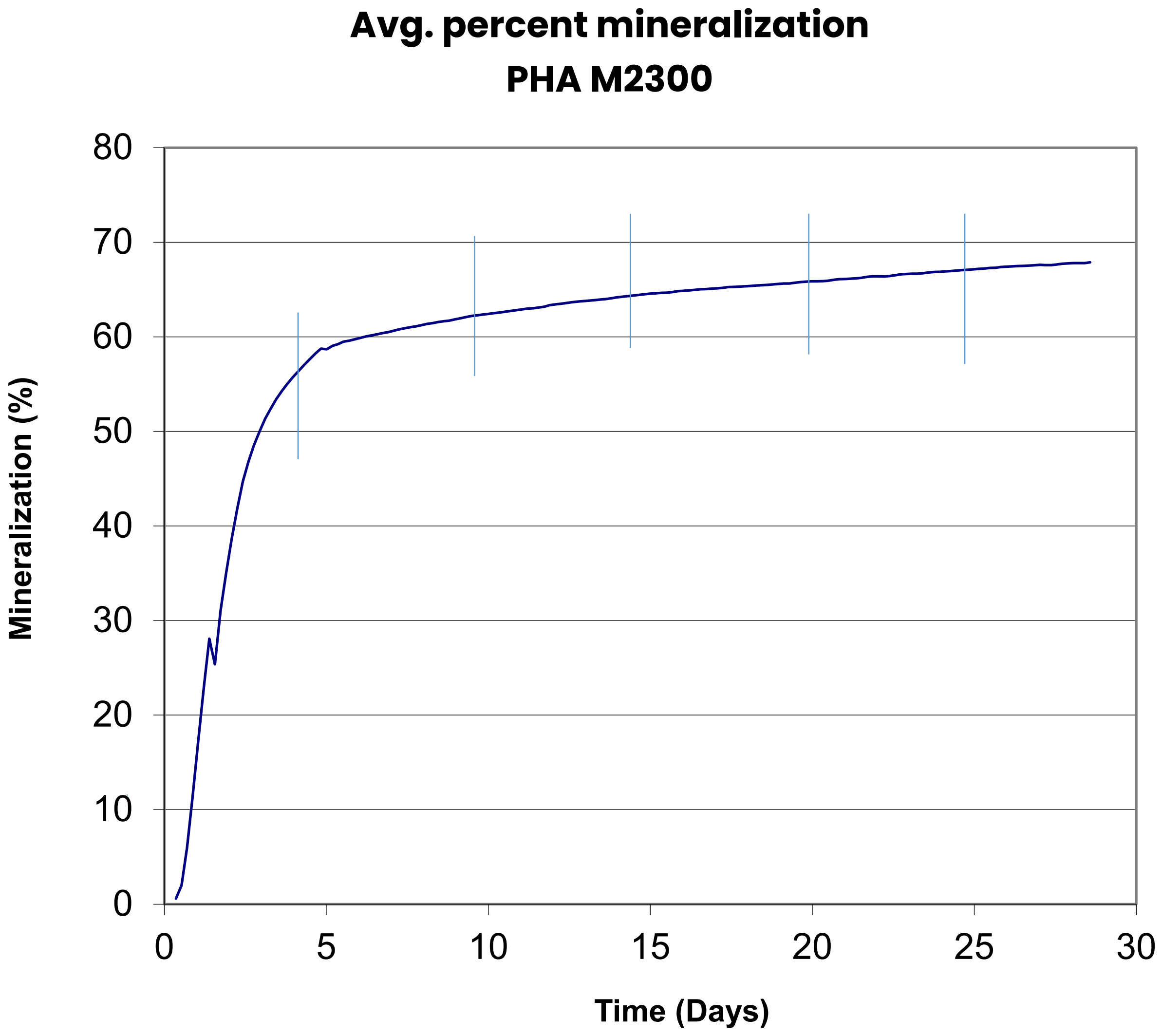
PHBV
Mineralization (%)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)



Negative Control
Evolution of C02 umol
1400
1200
1000
800
600
400
200
0
-200
CO2-19 -0.052
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (days)

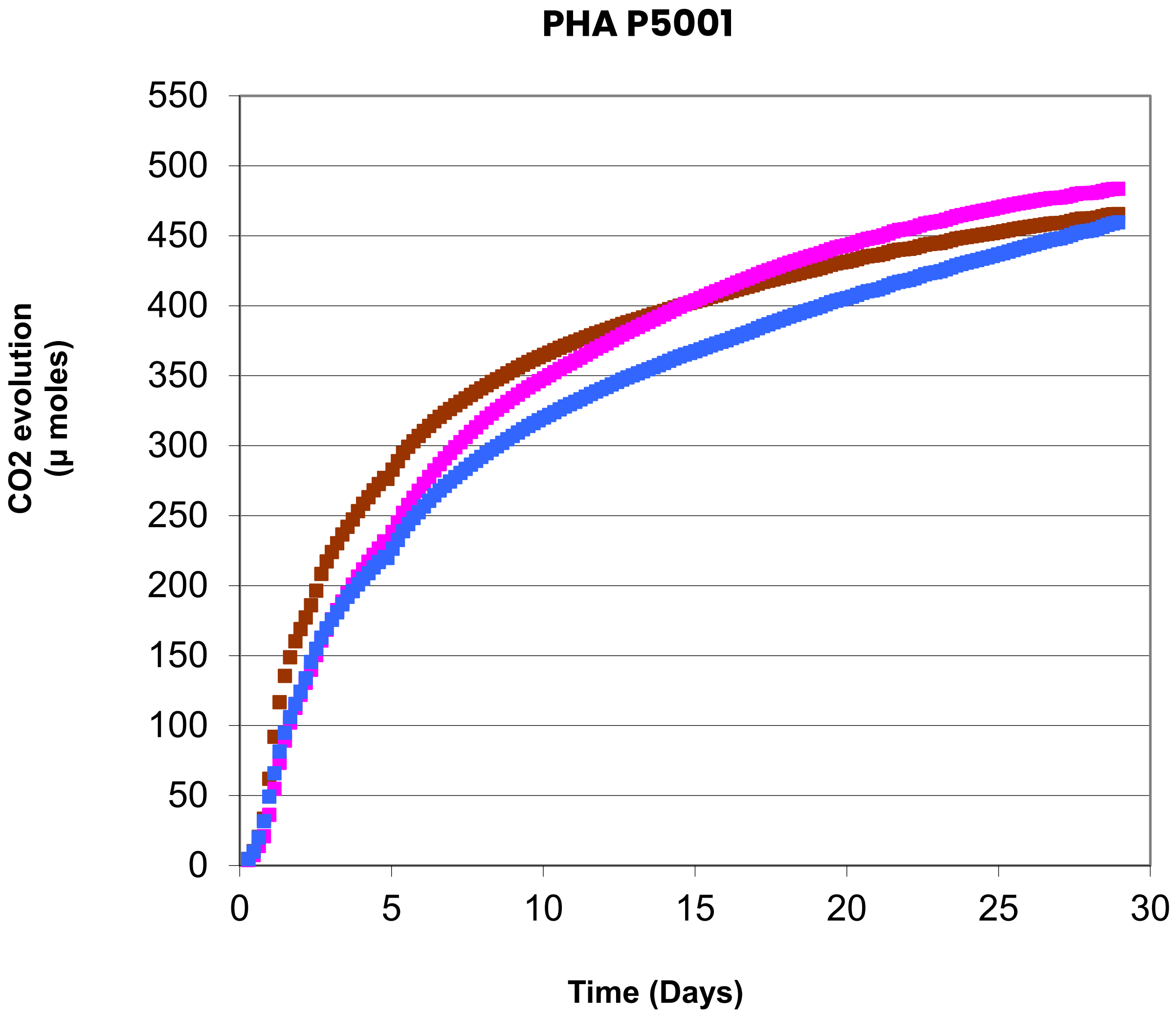
Tier I Marine Biodegradation Polyhydroxyalkanoate

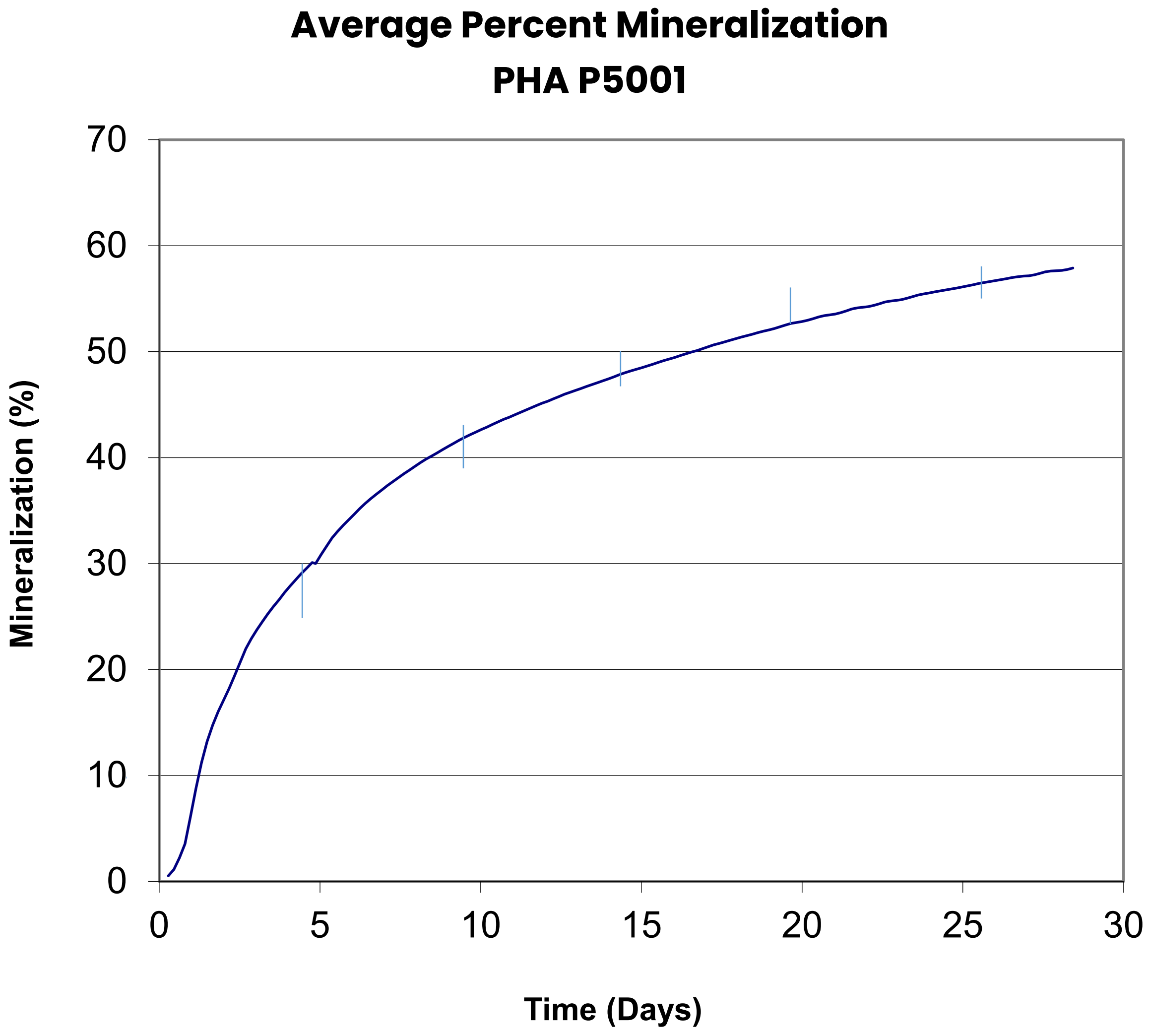




Tier I

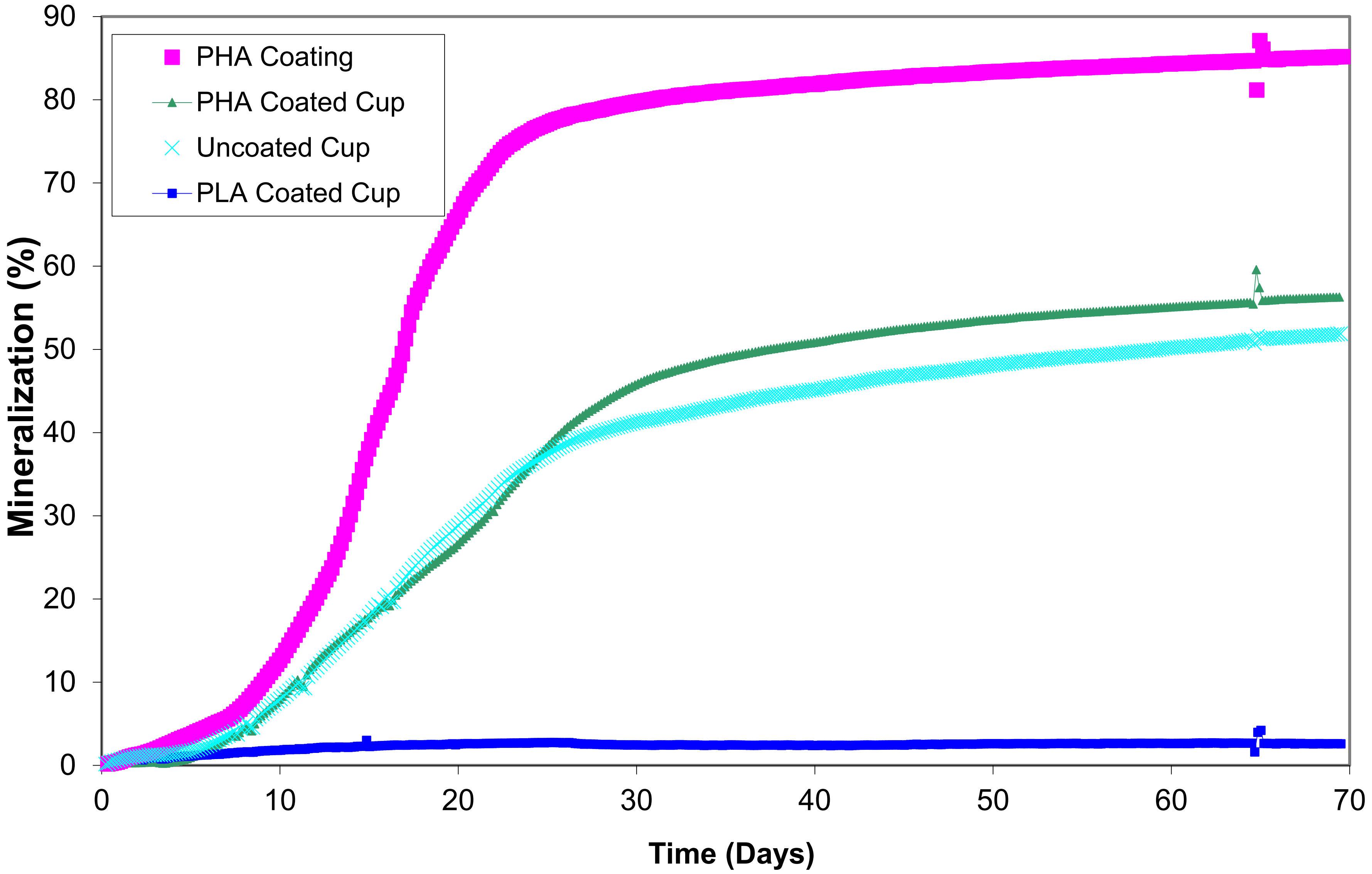
Marine Biodegradation Polyhydroxyalkanoate





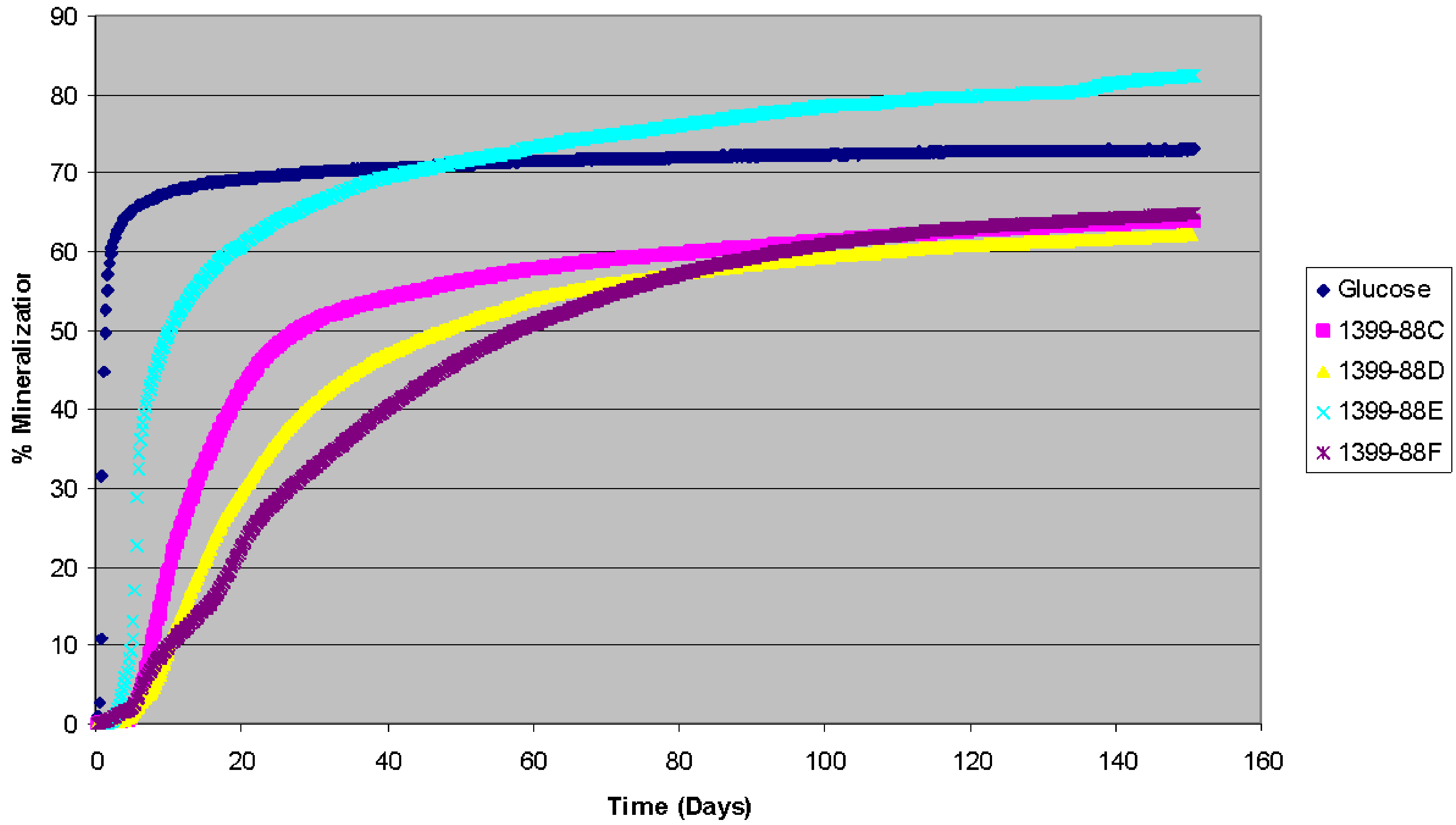
Tier I

Marine Biodegradation PHA Cups

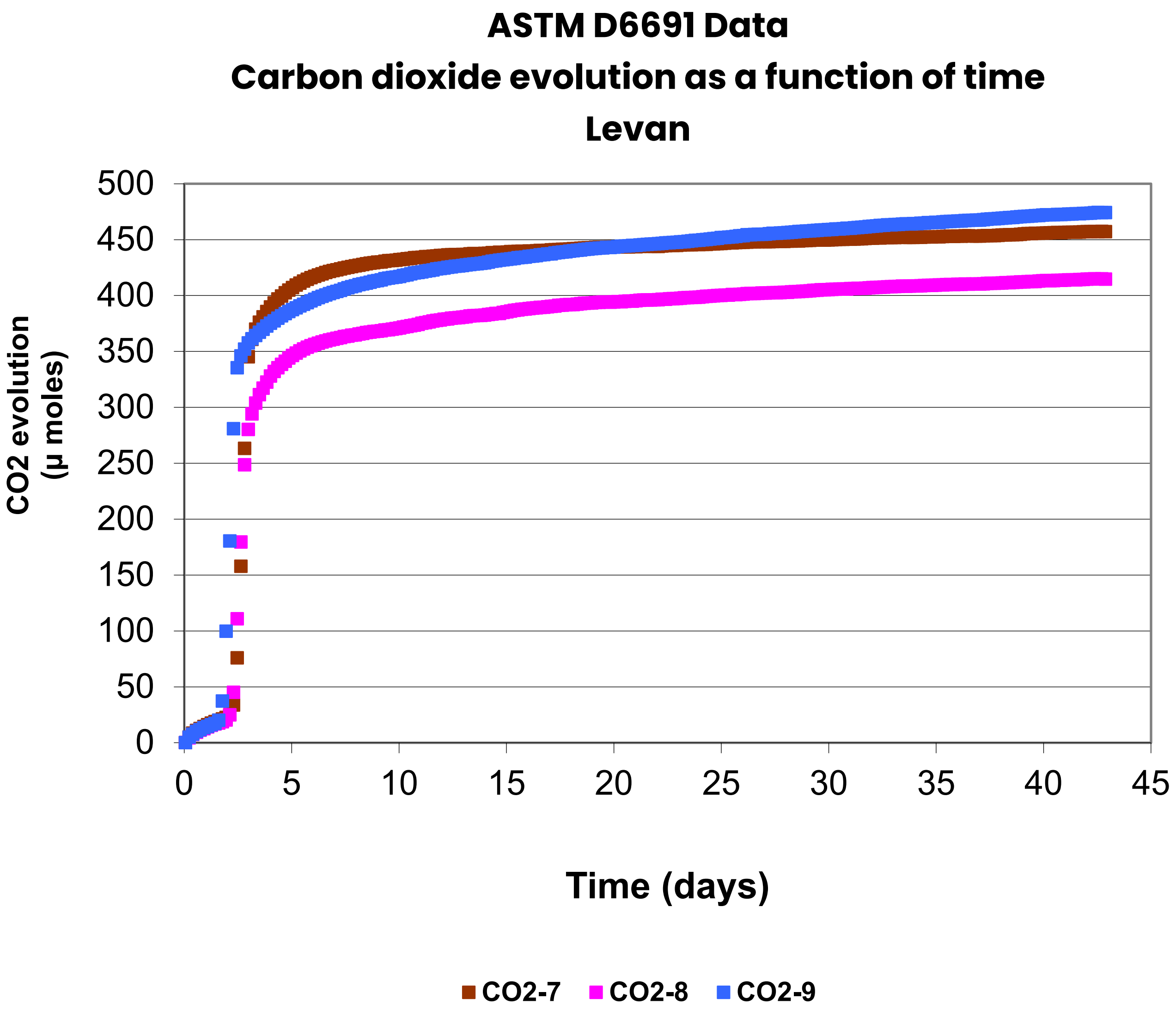


Tier I

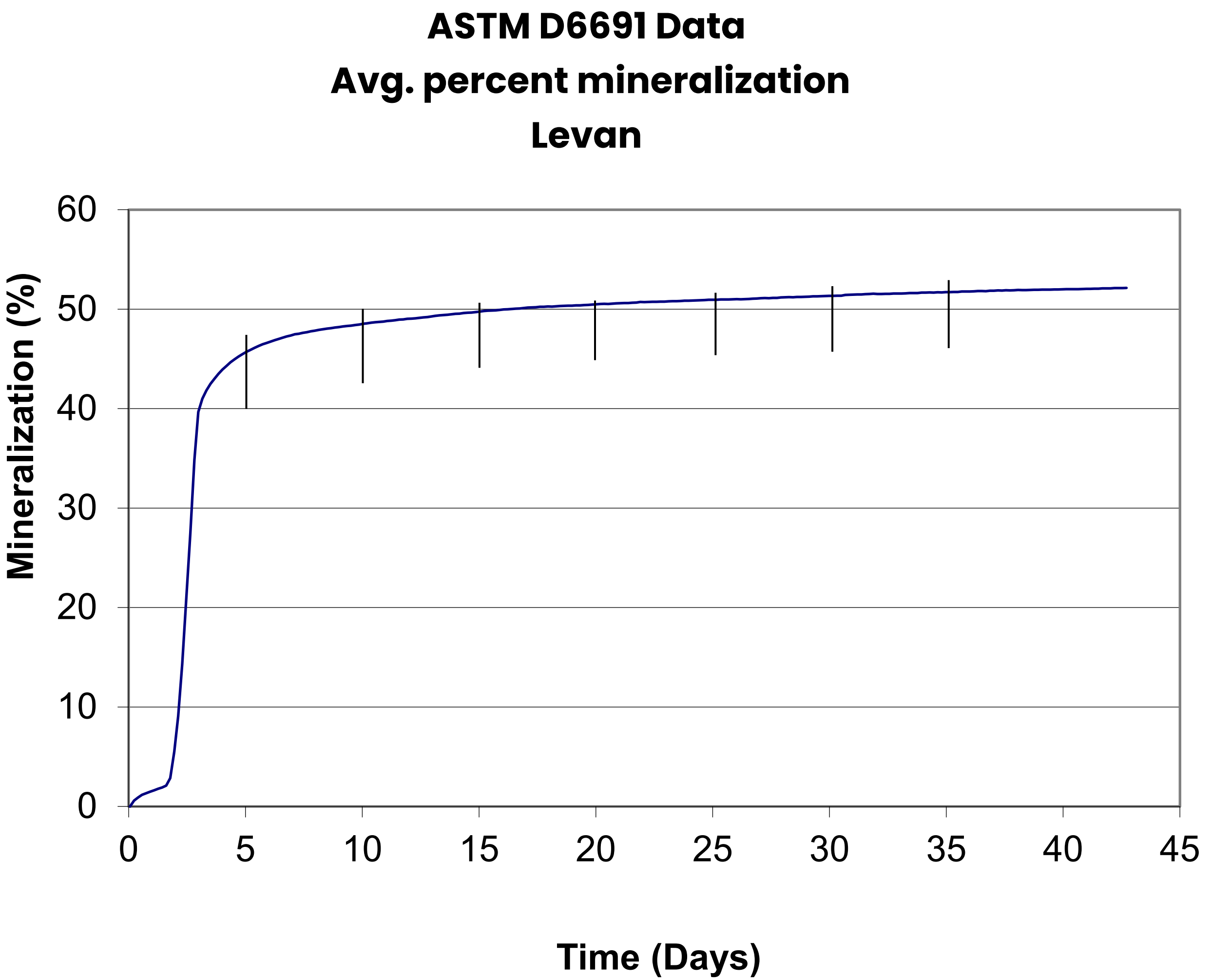
Metabolix 1399 Series – PHA Samples – ASTM 6691



Tier I Levan

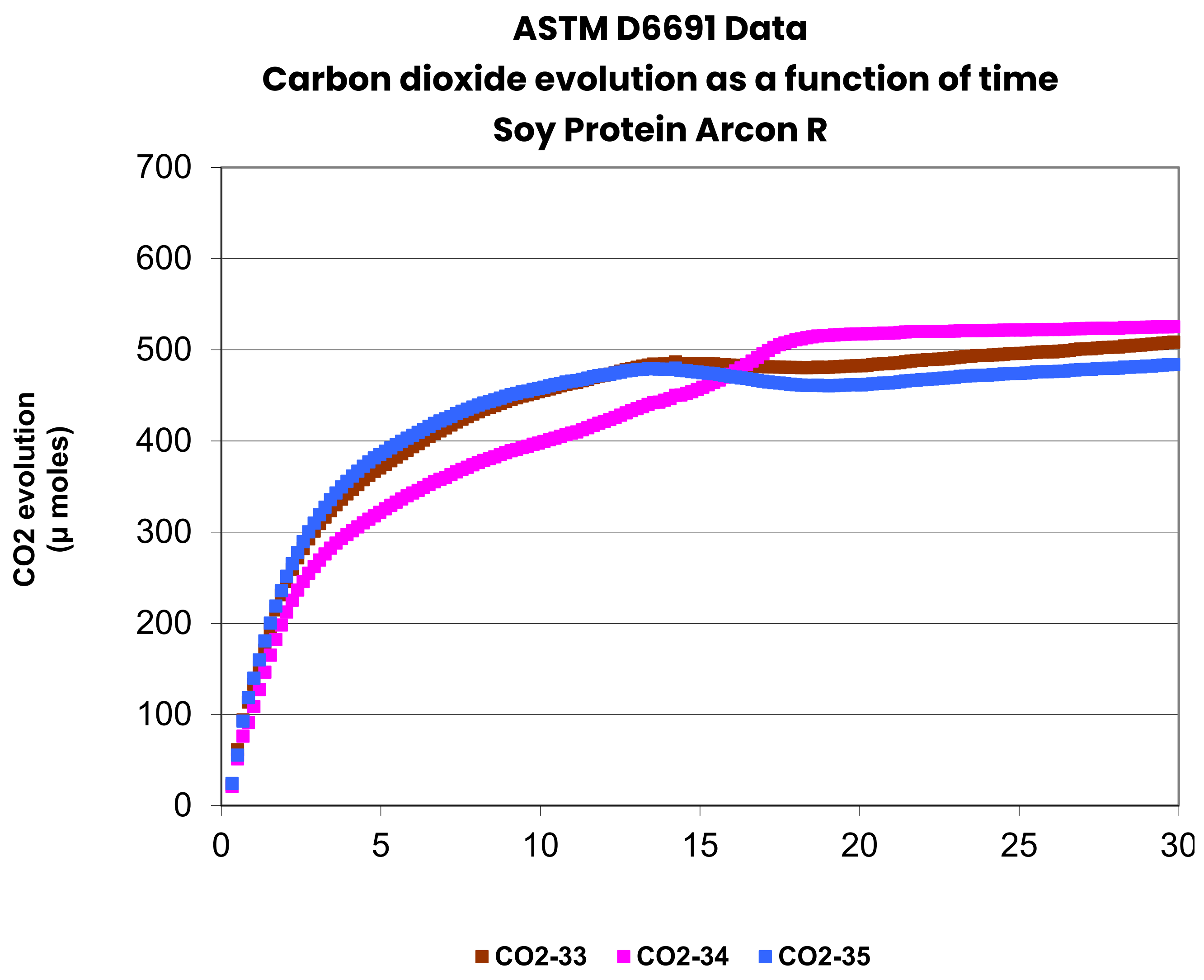


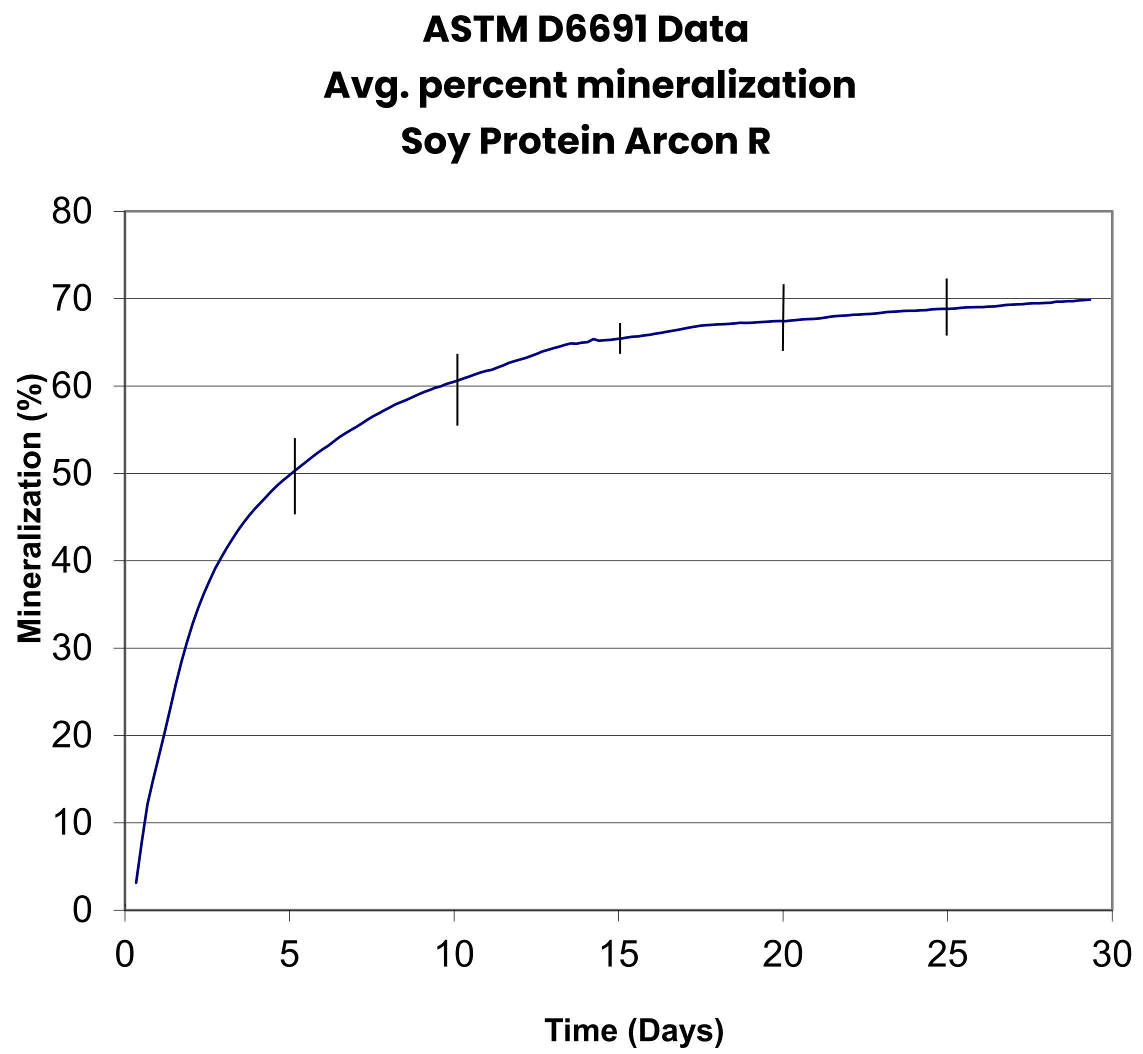
ASTM D6691 Data
Carbon dioxide evolution as a function of time
Levan
CO2 evolution
(μ moles)
500
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
Time (days)
CO2-7
CO2-8
CO2-9



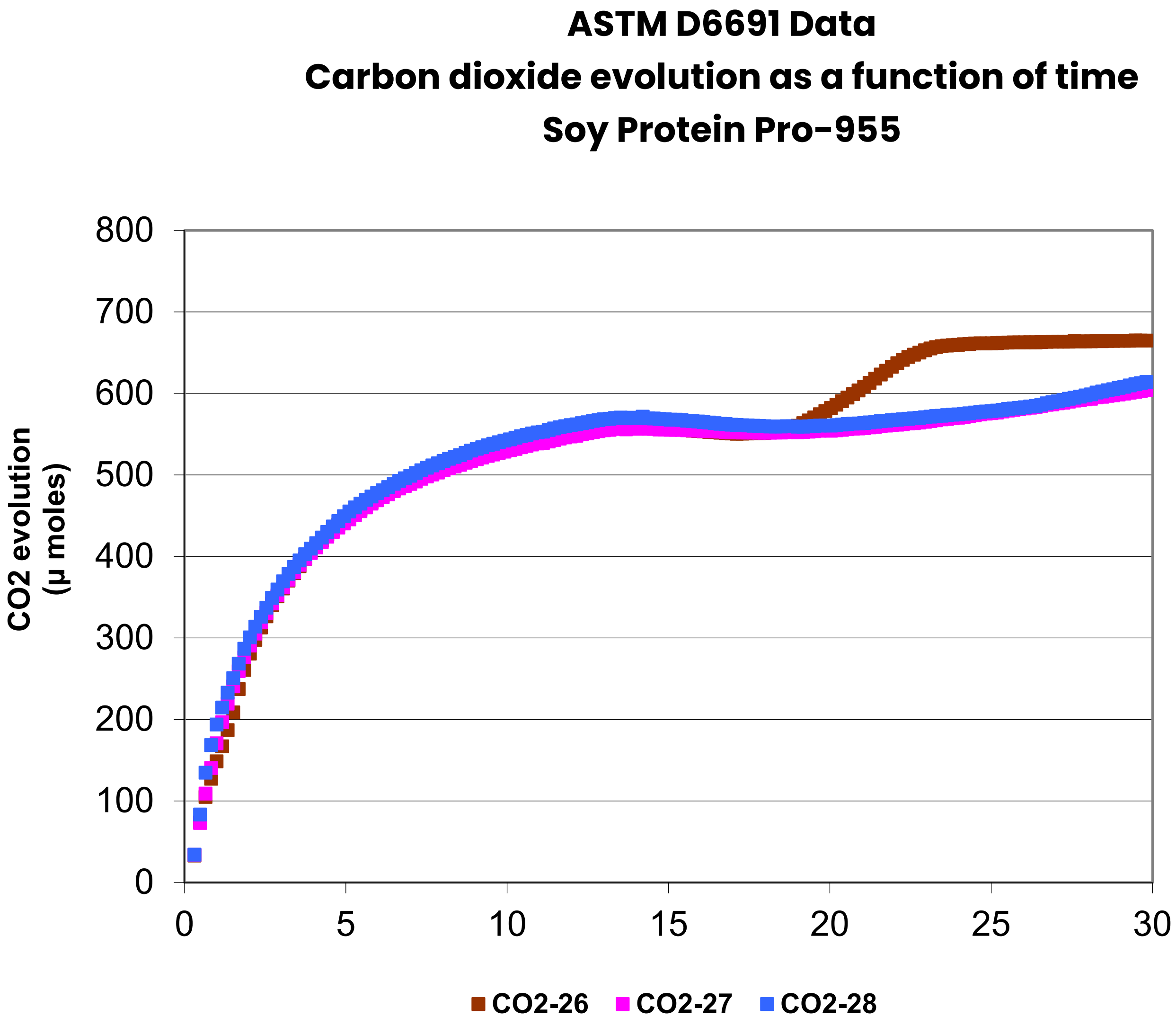
ASTM D6691 Data
Avg. percent mineralization
Levan
Mineralization (%)
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
Time (Days)

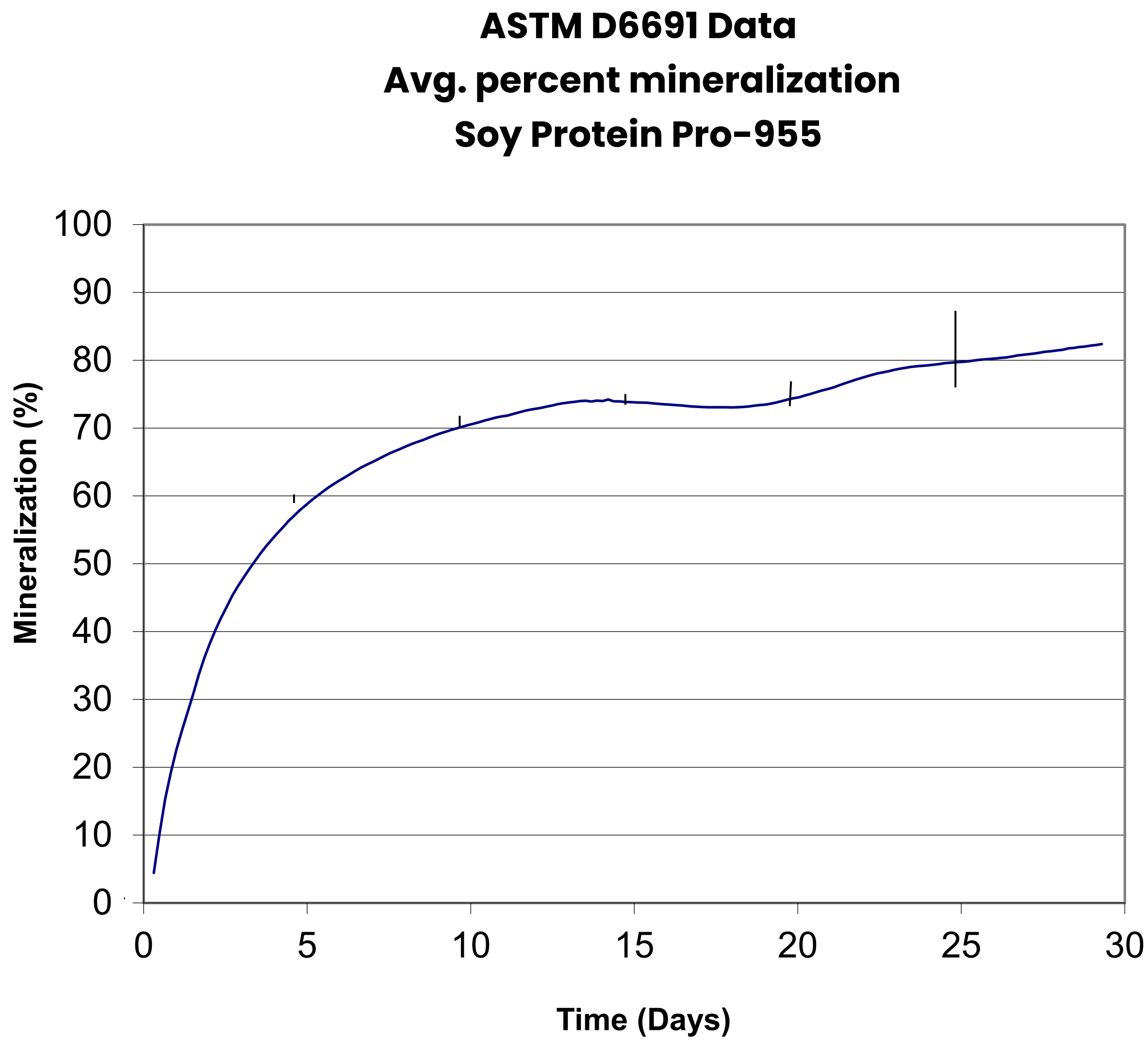
Tier I Soy Protein Arcon R





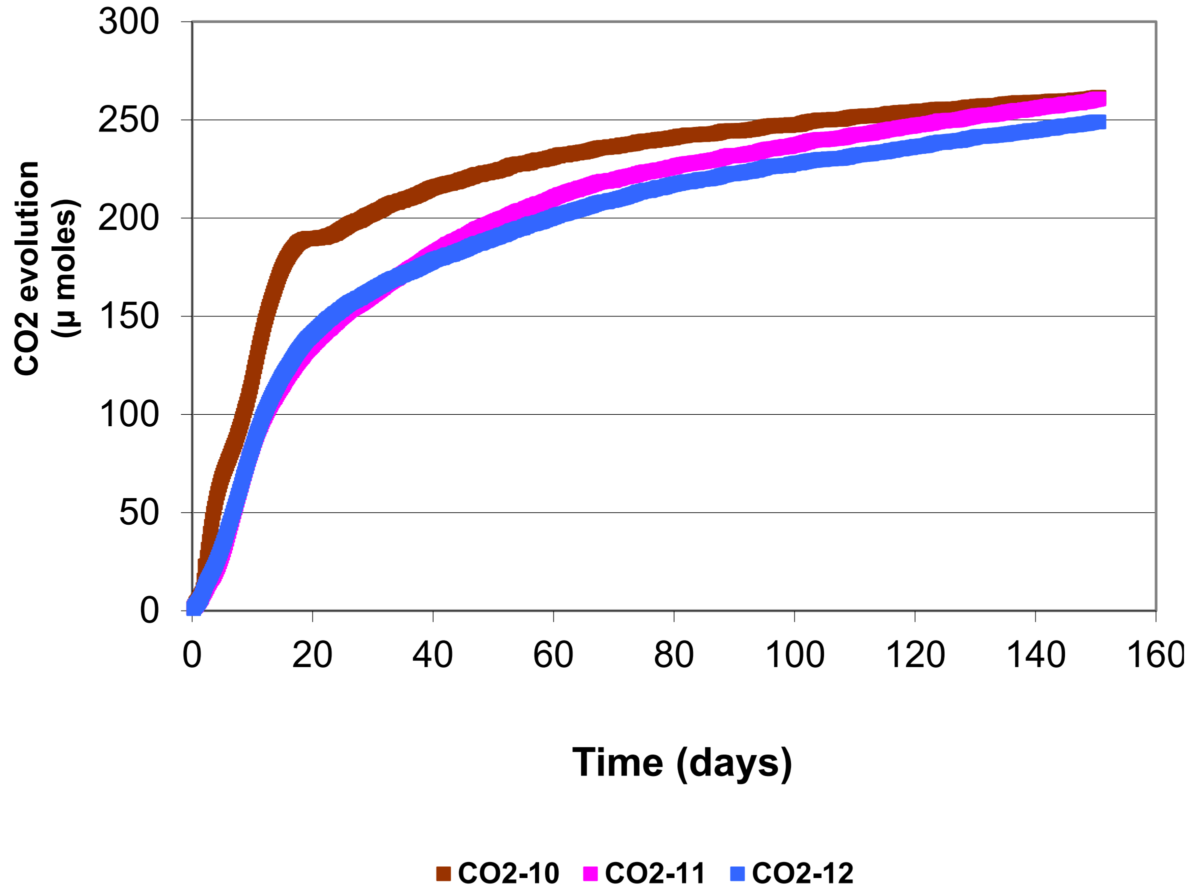
Tier I Soy Protein Pro-955



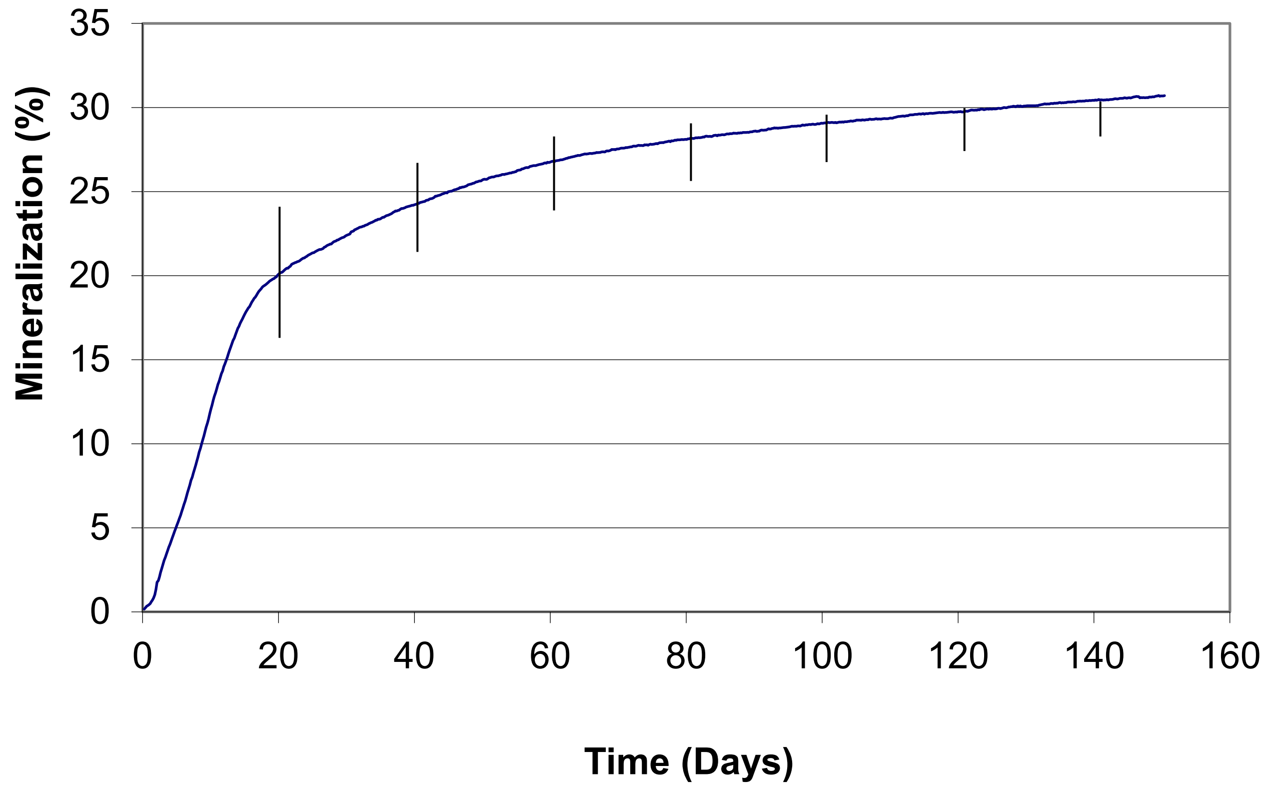


Tier I V3C

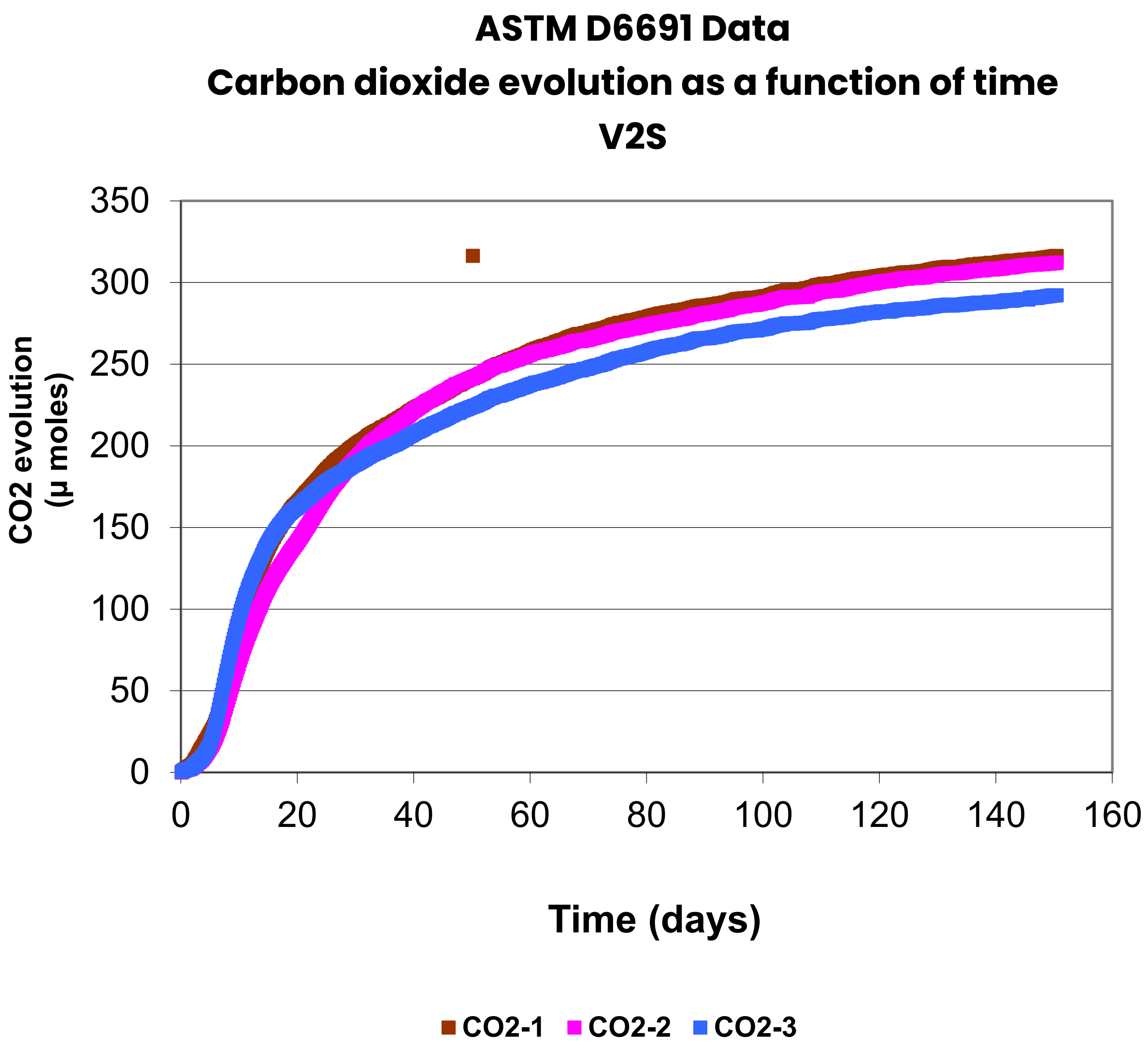
ASTM D6691 Data
Carbon Dioxide evolution as a function of time
V3C



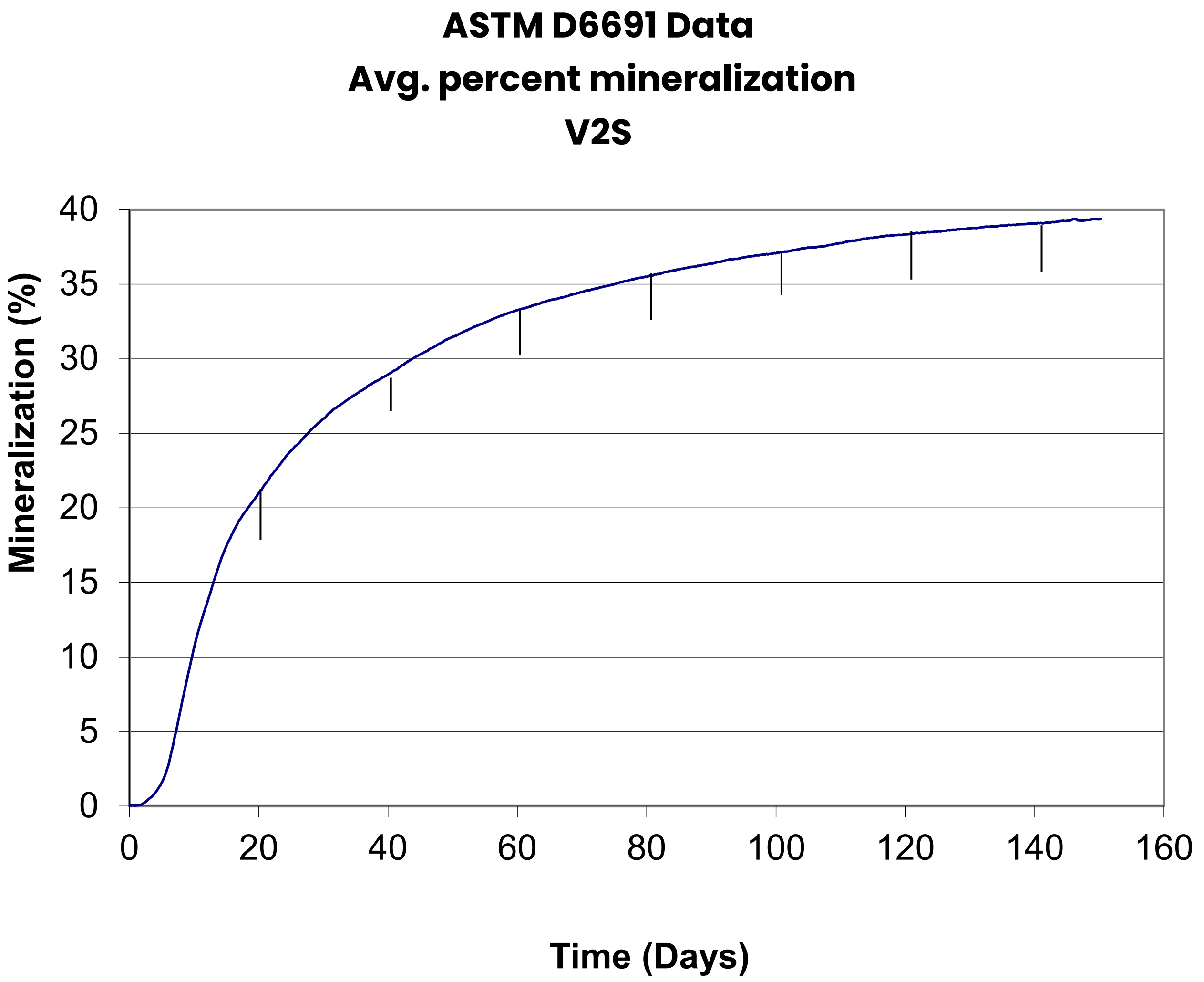
ASTM D6691 Data
Avg. percent mineralization
V3C



Tier I V2S



ASTM D6691 Data
Carbon dioxide evolution as a function of time
V2S
CO2 evolution (µ moles)
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
Time (days)
CO2-1
CO2-2
CO2-3



ASTM D6691 Data
Avg. percent mineralization
V2S
Mineralization (%)
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
Time (Days)

Tier II

Incubation Testing in the Laboratory

- 125 ml flask
- Sample size 1 x 1 inches
- 20ºC
- NSW Natural Sea Water
- NSW + sediment
- Nutrients nitrogen and phosphorous (0.5g/l nitrogen (NH_4Cl) and 0.1g/l phosphorous (KH_2PO_4)
- Shaken at 200 rpm
- Harvest at time points (7, 16, 26, 35, 47 days)
- Take dry weight – calculate weight loss/surface area

Tier II

Aquarium Incubations

- Conducted in tanks with continuously flowing seawater over 100 day period
- Harvest at time points (30, 50, 75, 100 days)
- Take dry weight – calculate weight loss/surface area
- Temperature Range 18-23°C
- pH 7.8-8.0
- Ammonia concentration .4 mg/l -.1 mg/l
- Phosphate concentration .075 mg/l -.01mg/l
- Two exposure conditions
- Contact with flowing seawater
- Contact with flowing seawater and sediment

Tier II

Aquarium Incubations

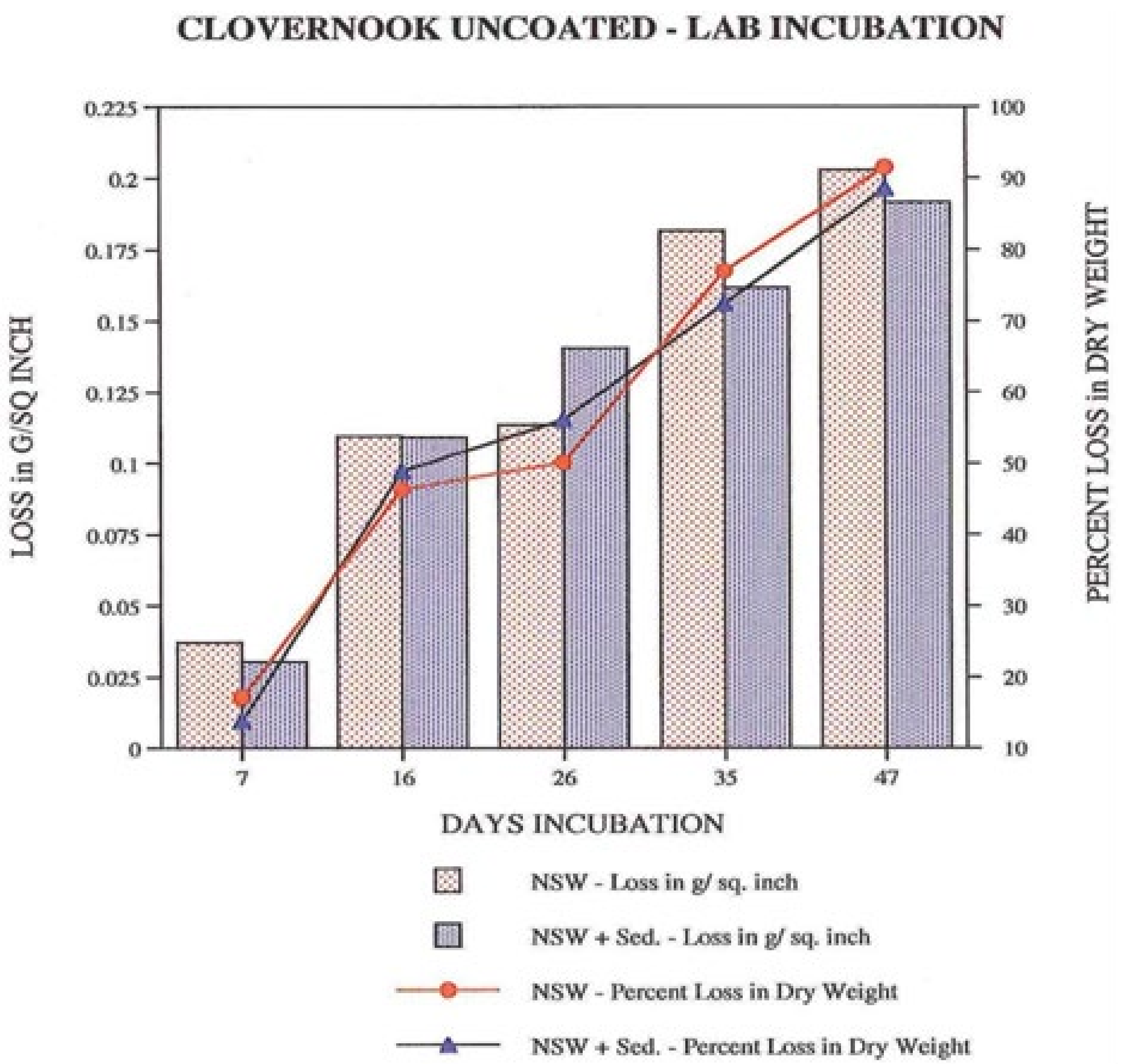
Laboratory

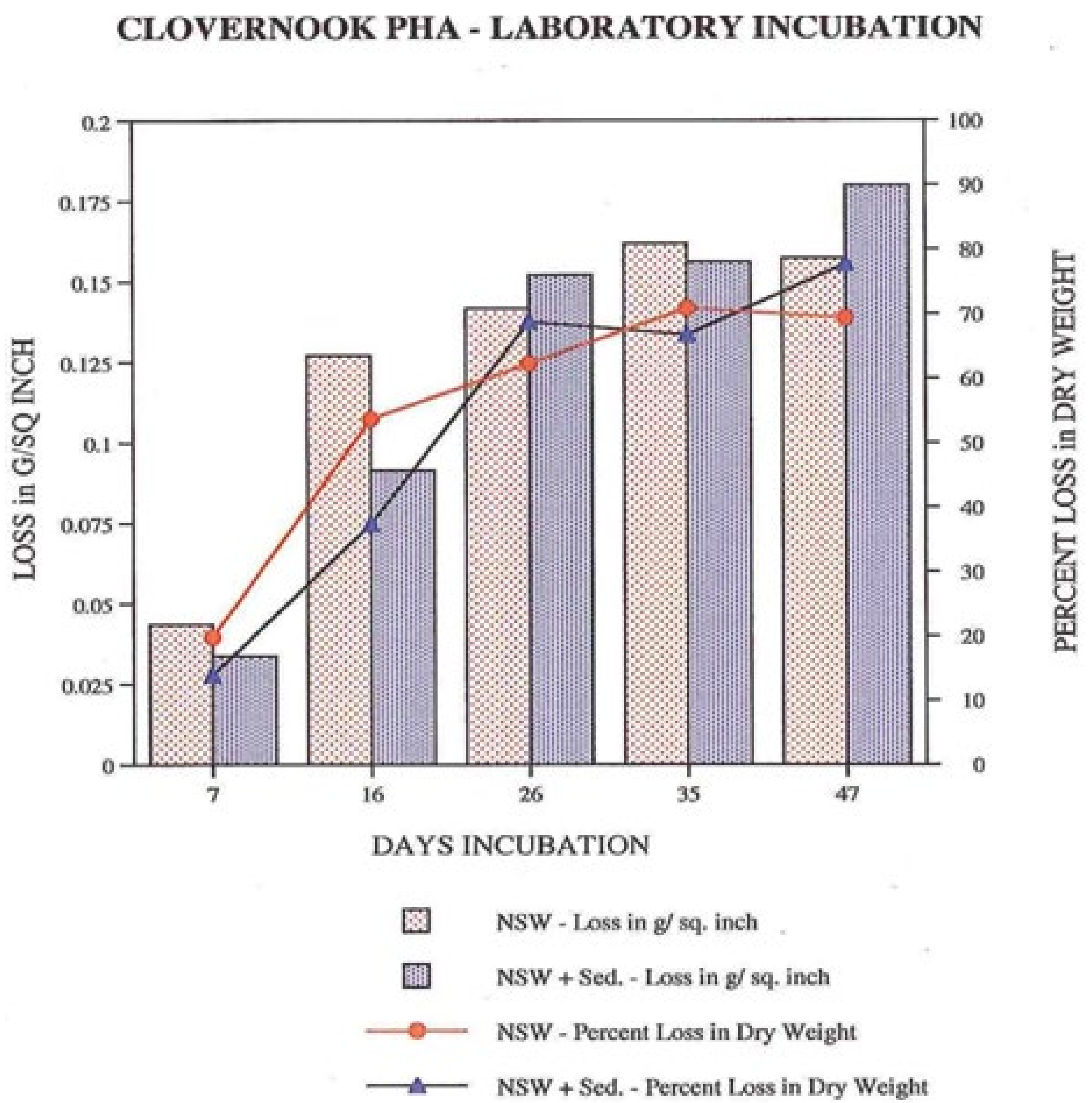
- Optimum conditions for biodegradation.
- Constant temperature.
- Excess nutrients.
- Continued aeration.

Aquarium

- Open system conditions.
- Flushing, removal of metabolic by-products, re-supply of oxygen.
- Season effects of temperature and nutrient supply variation.

Tier II Laboratory Incubations

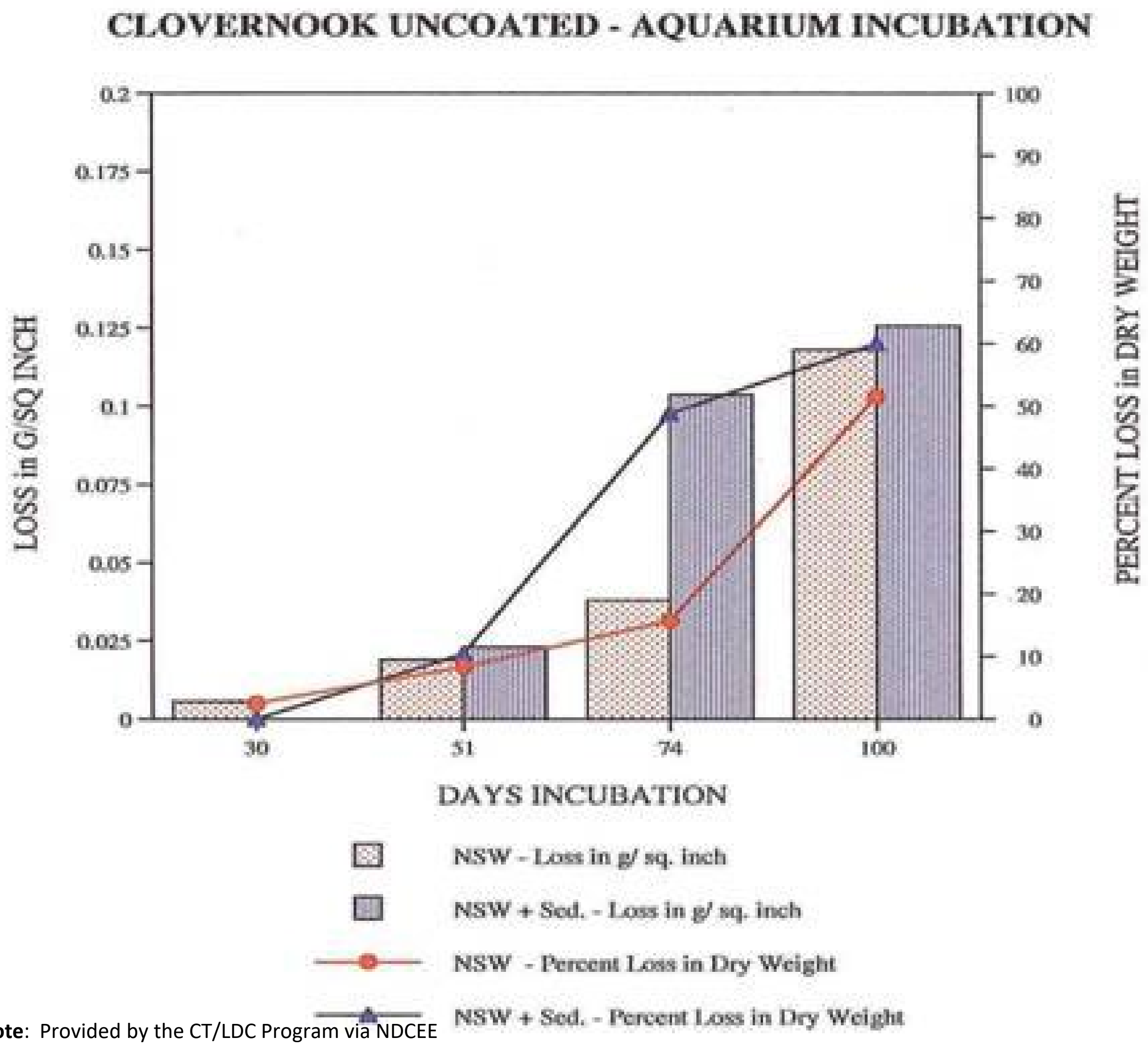


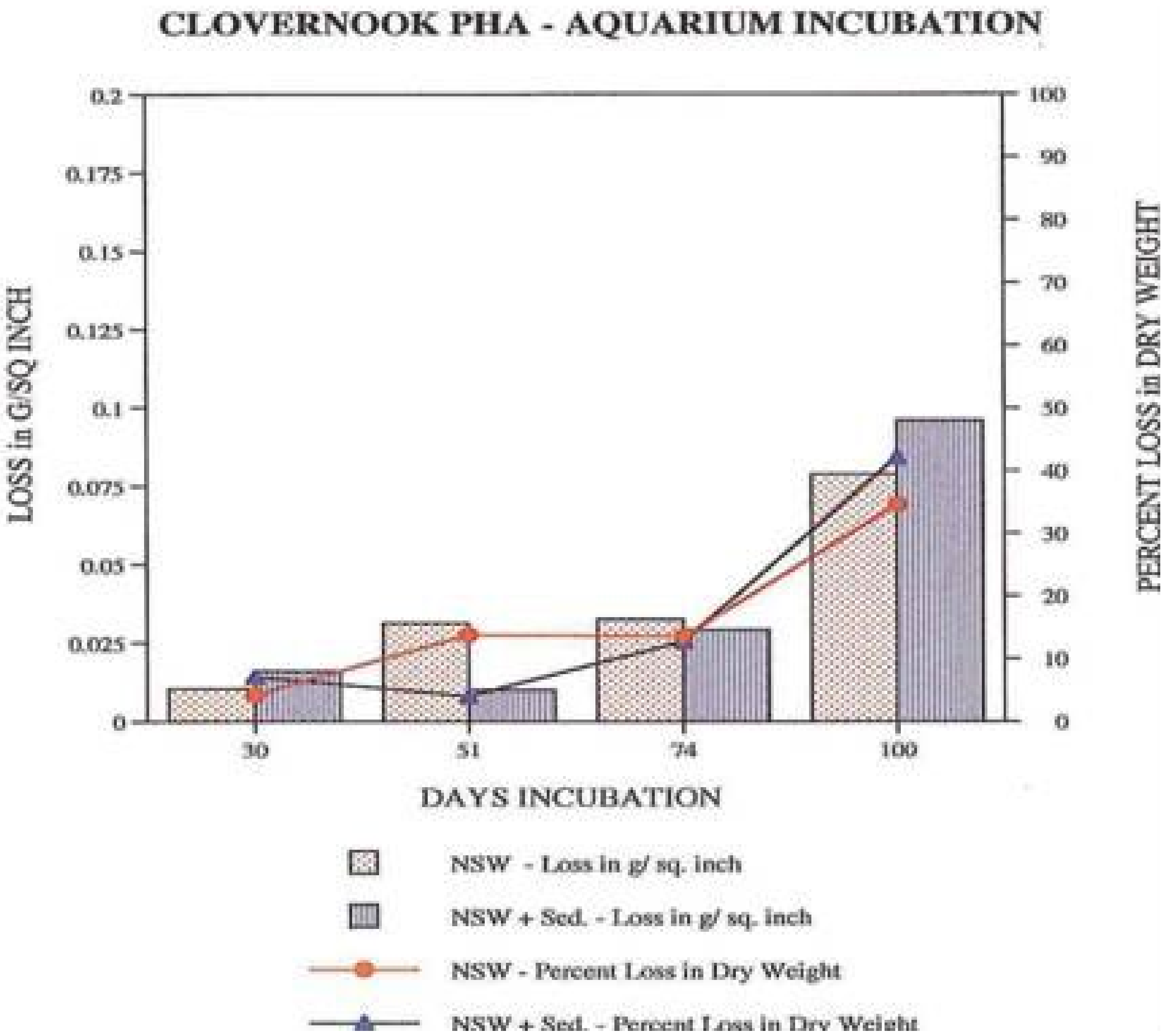


Note: Provided by the CT/LDC Program via NDCEE

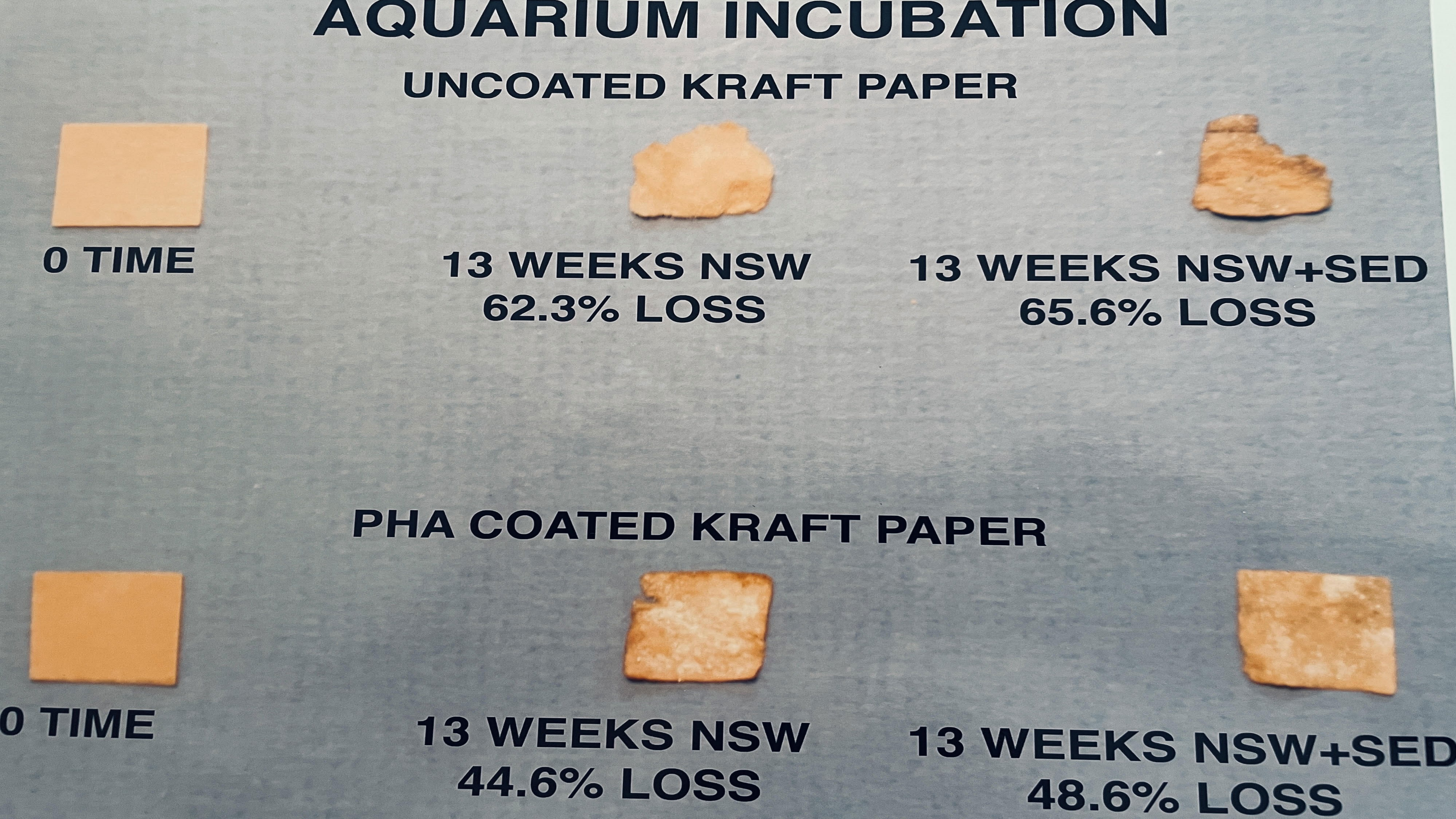
LABORATORY INCUBATION OF CUP SAMPLES AFTER 26 DAYS

Tier II Aquarium Incubations





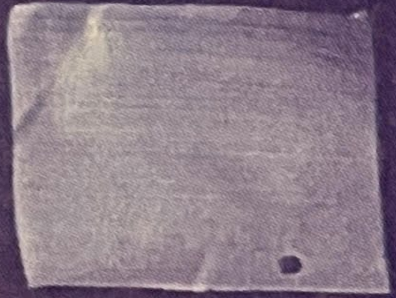
Note: Provided by the CT/LDC Program via NDCEE


AQUARIUM INCUBATION
UNCOATED KRAFT PAPER
0 TIME
13 WEEKS NSW
62.3% LOSS
13 WEEKS NSW+SED
65.6% LOSS
PHA COATED KRAFT PAPER
0 TIME
13 WEEKS NSW
44.6% LOSS
13 WEEKS NSW+SED
48.6% LOSS

Biodegradation of PCL in Aquarium Incubation

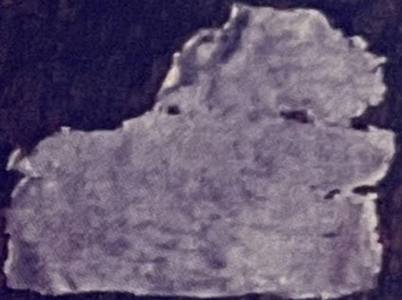
Natural Seawater

1 mo



3 mo

7 mo



Natural Seawater / Sediment Surface

1 mo

3 mo

7 mo

Tier II Testing of PHA Products in Interlaboratory Study

6 months seawater immersion weight 13.7g, 41% weight loss
9 months seawater immersion weight 3.7g, 78% weight loss
12 months seawater immersion weight 1.5g, 91% weight loss
15 months no sample remaining

6 month seawater immersion weight 2.2g, 70% loss
9 months seawater immersion no sign of phone unit

Tier III Coastal Field Incubations

Off shore

- Shallow water moorings 19 miles south west of Woods Hole (water depth 41 m)
- Plexiglass box with compartments – mesh screening
- Incubated up to 8 months

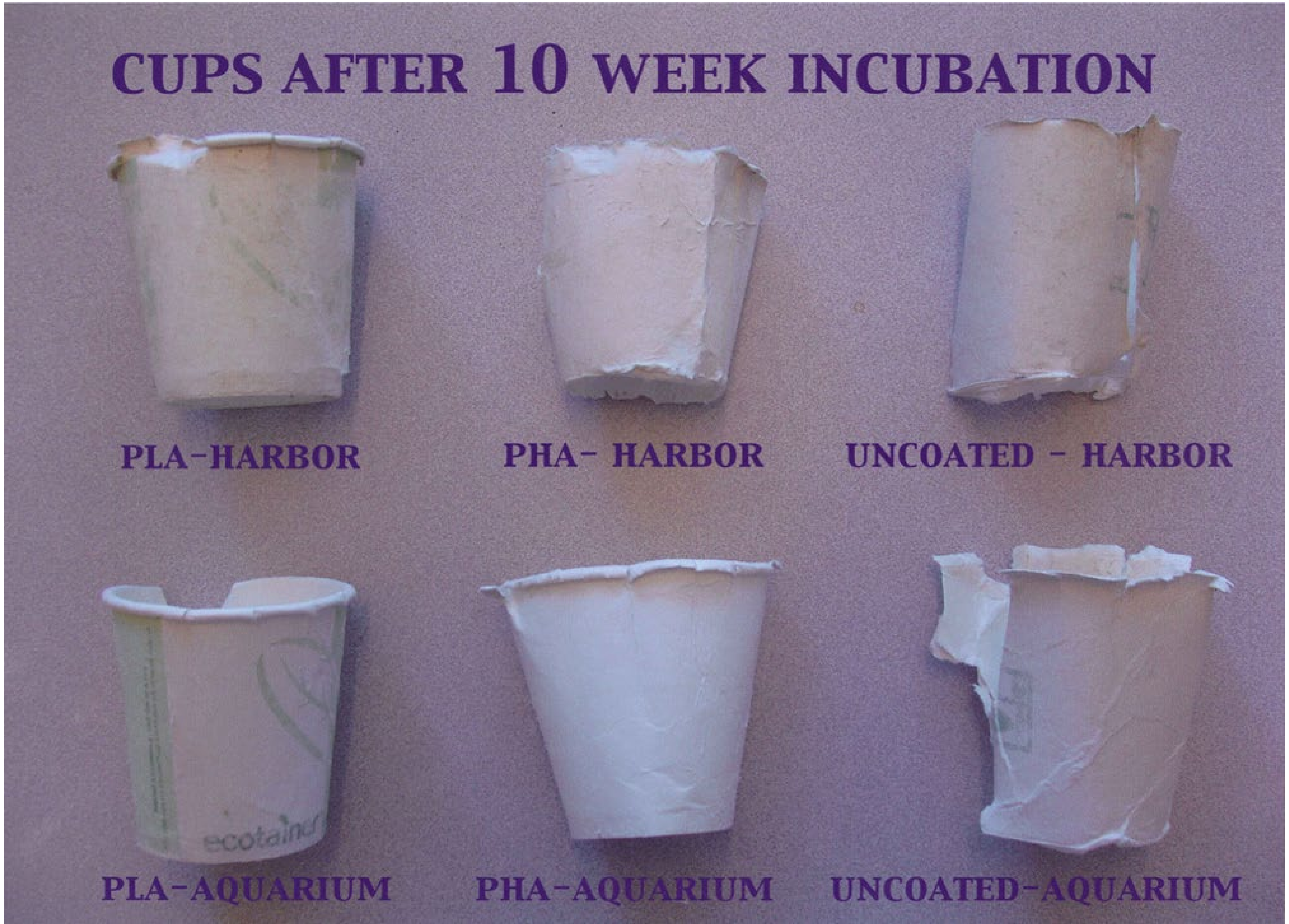
In shore

- Harbor incions
- Retrieved with SCUBA
- Covered mud boxes (water depth 22 m)
- Marsh grass (spartina alterniflora) exposure ubat

Deep Sea

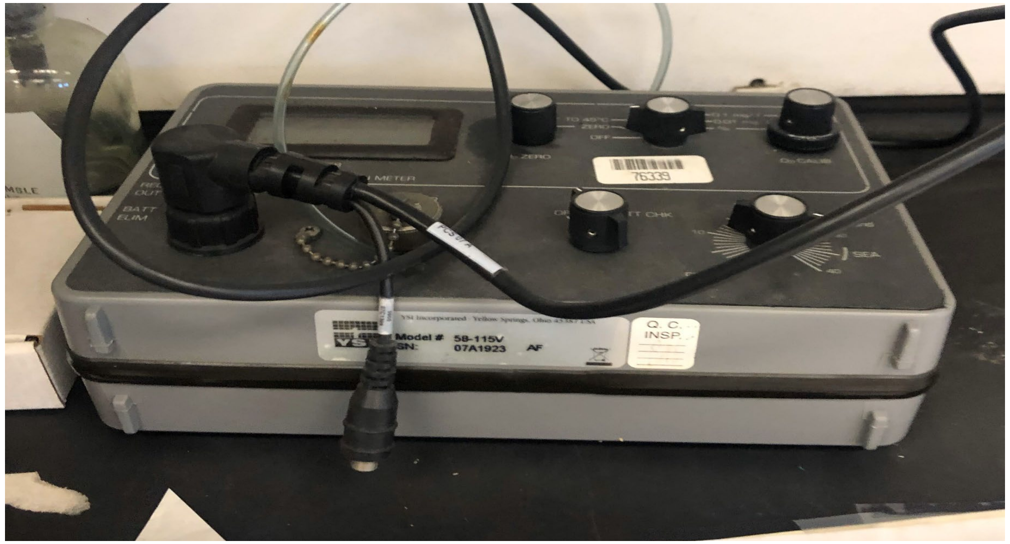
- Nylon mesh bags with 3 mm openings contain polymers
- Depth 4000-4500 m in Atlantic Ocean south of Bermuda (analyze as in Tier II)

Tier III


CUPS AFTER 10 WEEK INCUBATION
PLA-HARBOR
PHA- HARBOR
UNCOATED - HARBOR
ecotainer
PLA-AQUARIUM
PHA-AQUARIUM
UNCOATED-AQUARIUM

TOXICITY TESTING POLYTOX™

- EPA Approved Test for toxicity
- Performed on a variety of biodegradable polymers
- No toxicity for the coated cups

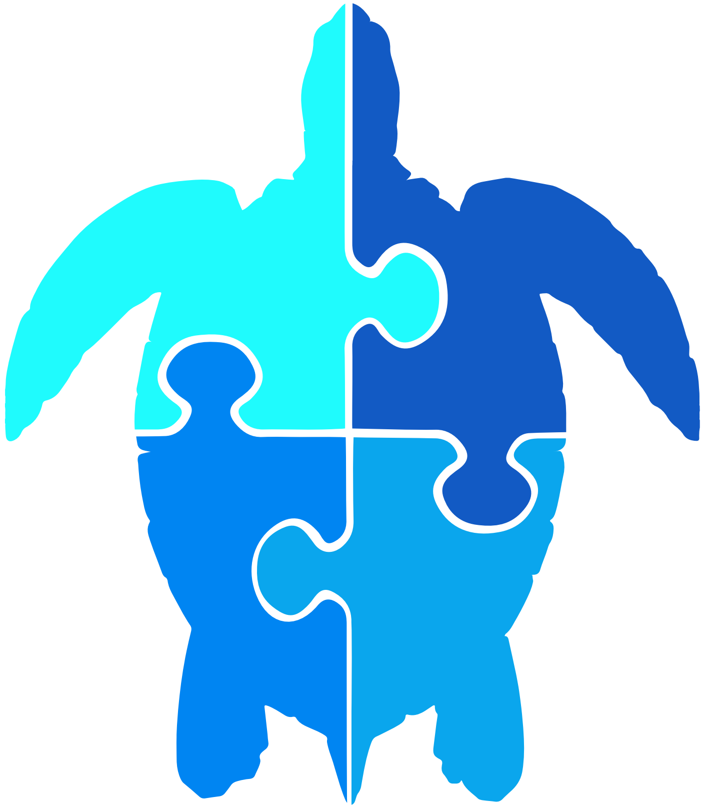


POLYTOX™ contains specialized microbial cultures that can determine the relative toxicity of water and wastewaters streams in about 30 minutes, with no expensive instrumentation required.

The respiration rate is the oxygen consumed by aerobic and facultative bacterial cultures and is expressed as mg of O2 consumed per liter per minute.

Challenges For Biodegradables

- Despite the MARPOL Treaty rules of no plastics overboard into the ocean, plastics are in the ocean.
- Biodegradable plastics cannot be currently disposed in the ocean (MARPOL Treaty) because they are a "plastic"
- Inability to reach consensus on long-term impact of biodegradable materials
- Commercial availability of marine degradable polymers is limited
- Cost of biodegradable polymers are not favorable compared to petroleum-based resins




Do your part - Get Involved
Educate others and yourself
Promote Sustainability
What can you do?

Dedicated to and forever grateful to: Bill Buzzi and Teresa Clark

Thank You

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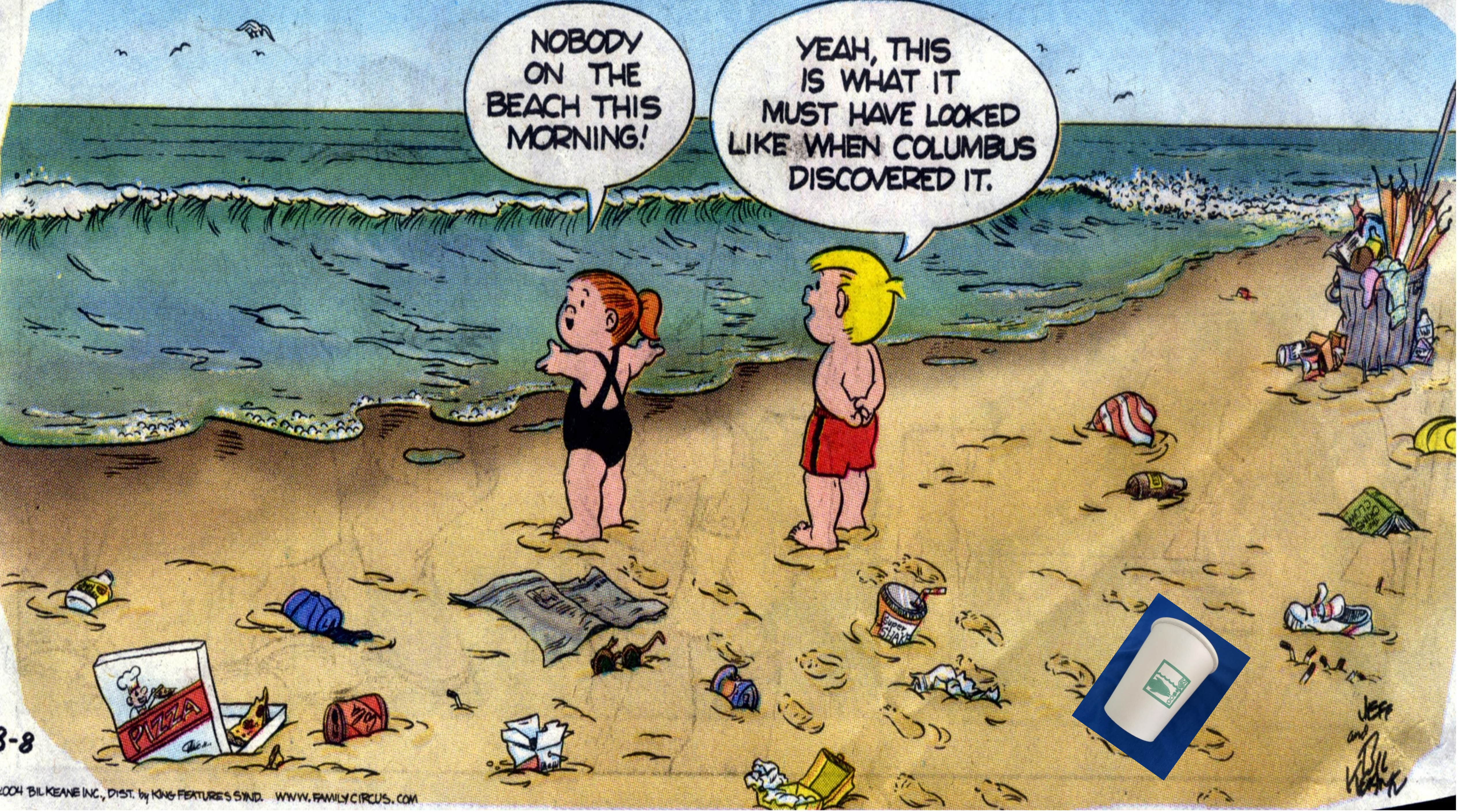
Dr. Robert Whitehouse

Questions


NOBODY ON THE BEACH THIS MORNING!
YEAH, THIS IS WHAT IT MUST HAVE LOOKED LIKE WHEN COLUMBUS DISCOVERED IT.
PIZZA
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Remember when we did this with shells???