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

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MASSACHUSETTS TECHNOLOGY COLLABORATIVE

COLUMBUS INSTRUMENTS

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University of Massachusetts Dartmouth SMAST

About Me

Dr. Micheline Labrie

Current Position

Research Assistant Professor,

Department of Estuarine and Ocean Sciences

Science Lead, Biodegradability Laboratory

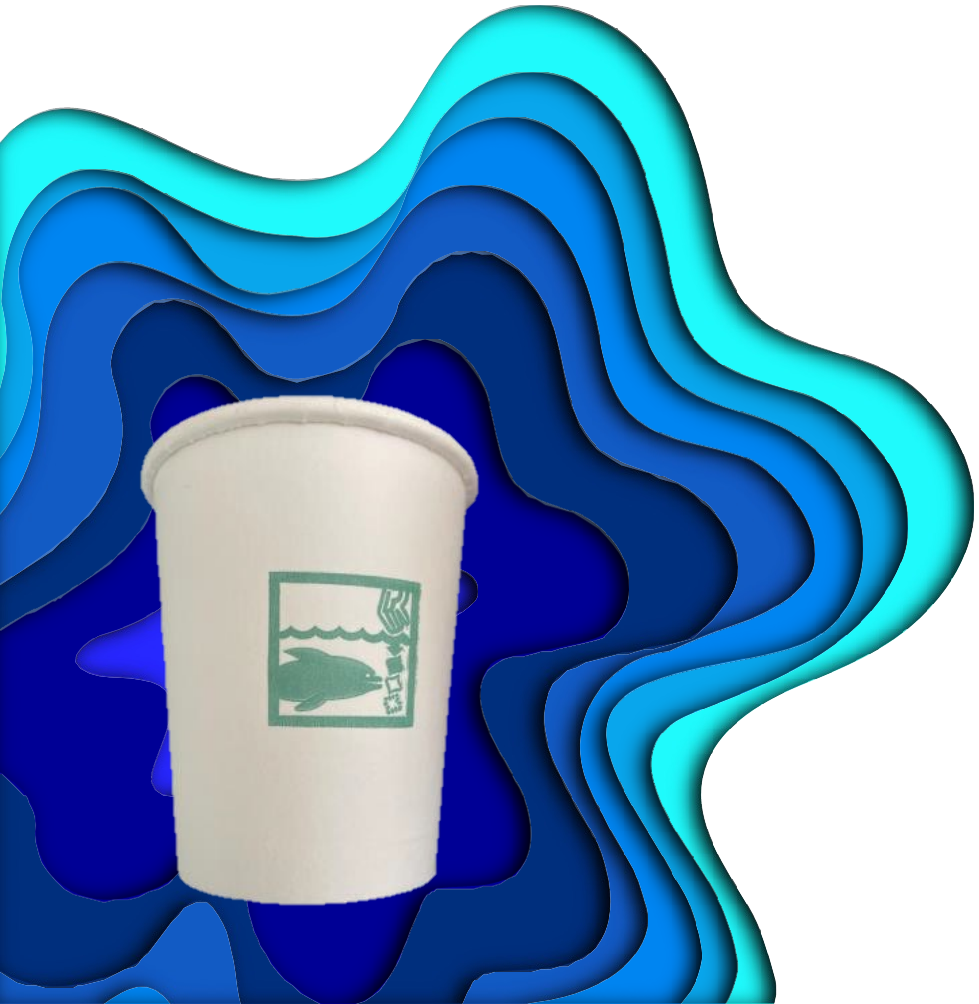
Researcher, Coastal Systems Program

Background

Biogeochemistry

Nutrient cycling in coastal systems

Outline



* University of Massachusetts Dartmouth Facilities
  - School for Marine Science and Technology
  - Establishment of the Biodegradability Lab
* Biodegradability Laboratory
  - Biodegradation overview
  - ASTM Standard Methods
  - Instrumentation
    - Micro-Oxymax Respirometers (4 systems)
* Testing for Biodegradable Polymers
  - Tier 1 Methods for biodegradation
  - Measuring environmental conditions
  - ASTM D6691 variables to consider
* Next Steps
  - Validation experiments

Questions for Panel


Maine
Vermont
Portland
New
Hampshire
Concord
Portsmouth
Massachusetts
Boston
Springfield
SMAST
Providence
New
York
Connecticut
New Haven
Rhode
Island
NYC


UNIVERSITY OF MASSACHUSETTS DARTMOUTH
MARINE CAMPUS

BIODEGRADABILITY LABORATORY

PRIMALOFT® BIO™

MASSACHUSETTS TECHNOLOGY COLLABORATIVE

Mass Tech Collaborative & PrimaLoft investments: >$1.1M

1. Increase Biodegradability Testing Infrastructure

1. Expand testing resources for industry needs. Quality control and product development

2. Accelerate Product Development

1. Screening tests like ASTM D6691

3. Establish Core Facility

1. Open lab for internal and external use.
    1. Research applications beyond biodegradable materials
2. Implement recharge rates for services

NEWS & PRESS RELEASES // 2021 // BAKER-POLITO-AWARDS-UMASS-DARTMOUTH-BIODEGRADABLE-PLASTICS-LAB

UMass Dartmouth awarded nearly $1.2M for new Biodegradable Plastics Lab

Baker-Polito Administration awards $700k, PrimaLoft gives $450K in public-private investment to boost new marine tech research facility for the South Coast

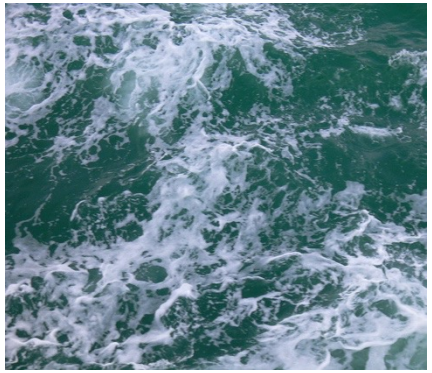
BIODEGRADATION

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

Terminology:
Polymer (to be tested) will be referred to as either the control or experimental substrate.

ASTM STANDARD METHODS



ASTM D6691
Seawater
aerobic

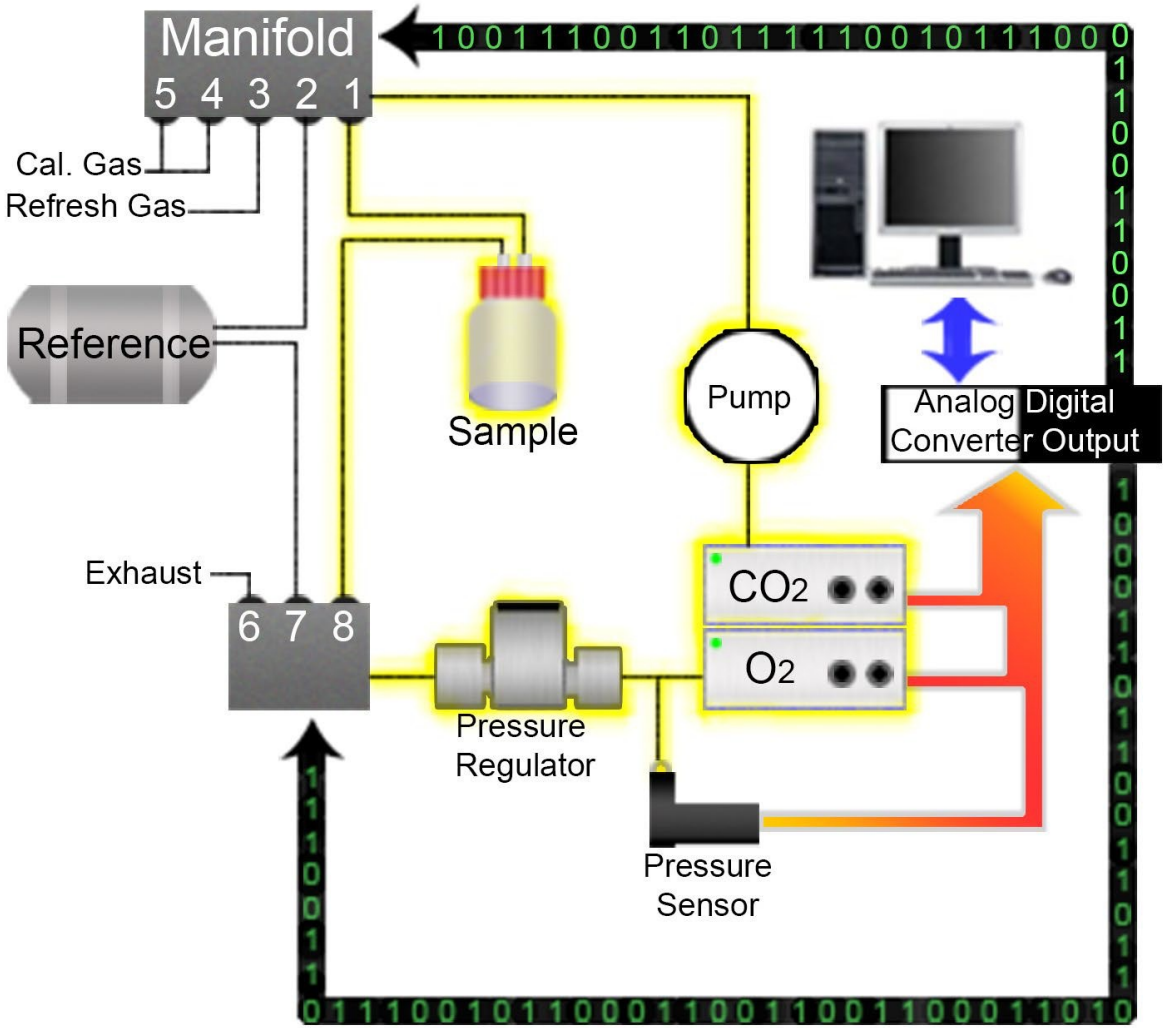
ASTM D5511
High-solids
anaerobic

ASTM D5210
Wastewater
anaerobic

ASTM D5338
Compost
aerobic

ASTM D5988
Soil
aerobic

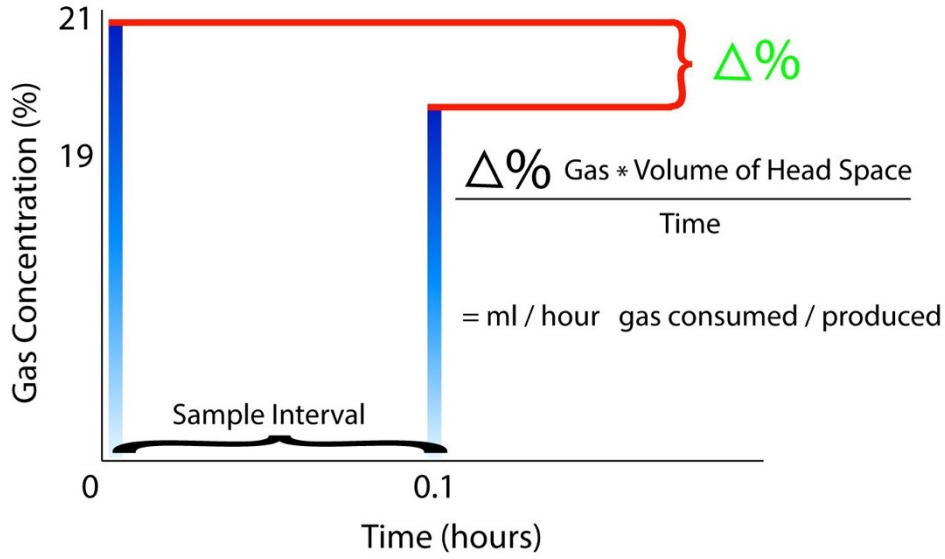
Micro-Oxymax Closed-Loop **Measurement Method**



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

...where:

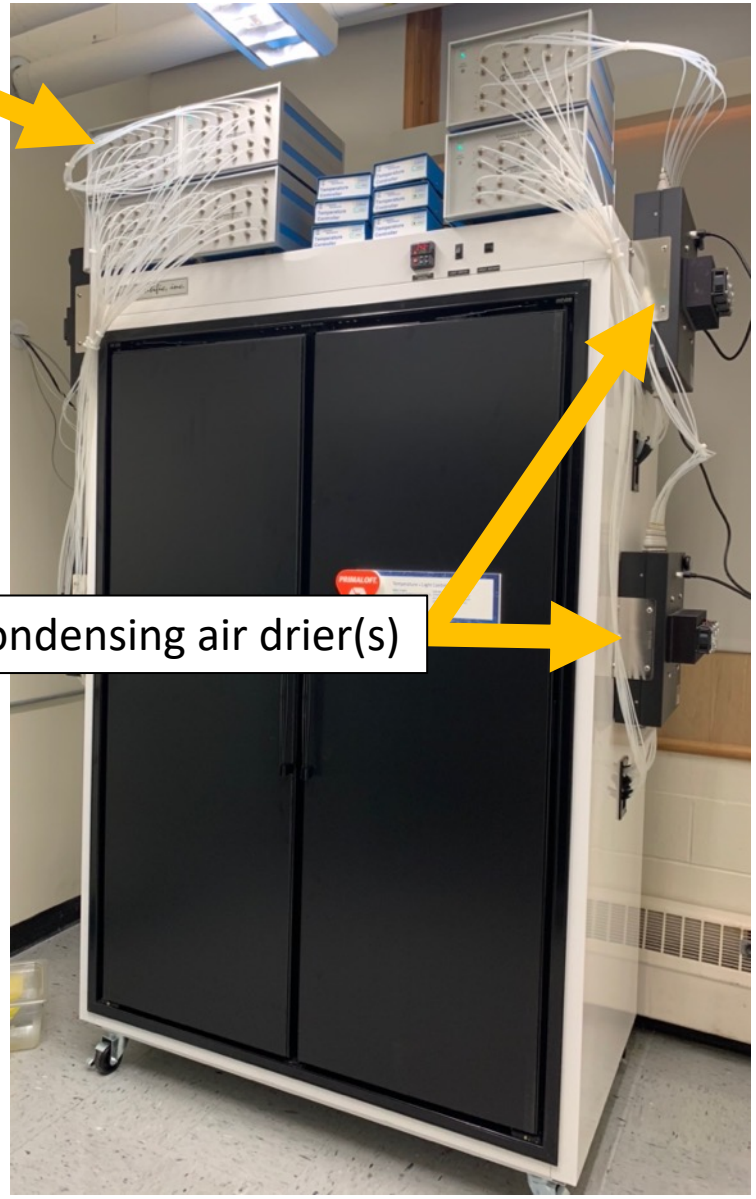
V_T = Sample Chamber Volume
V_S = Sensor Volume
V_R = Reference Chamber Volume
P_A = Barometric Pressure
1 = a remainder from the cancellation of units



Micro-Oxymax 60 Channel System (ASTM D6691)

- Closed-loop system
- Aerobic
- 0-3% CO_2 Non-dispersive infrared detector
- Condensing air driers
- Two orbital shakers

The Micro-Oxymax Respirometer is a highly sensitive instrument with a maximum sensitivity of 0.2 uL/hour rate calculation



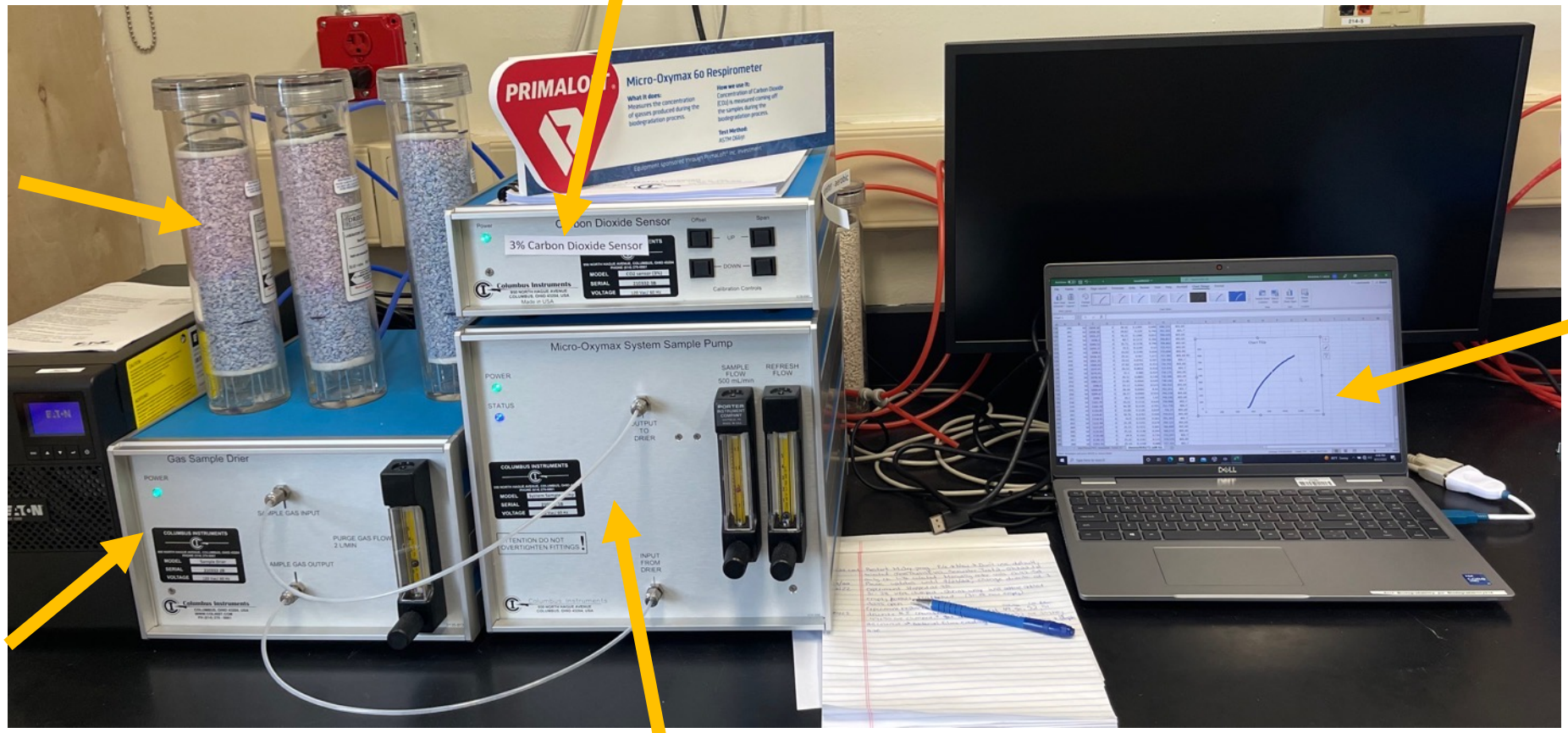
Temperature and light controlled incubator

Orbital shaker with 250 ml reactor vessels

Micro-Oxymax 60 Channel System (ASTM D6691)

0-3% sensor range CO_2 (Non-Dispersive Infrared Detection)

Drying columns



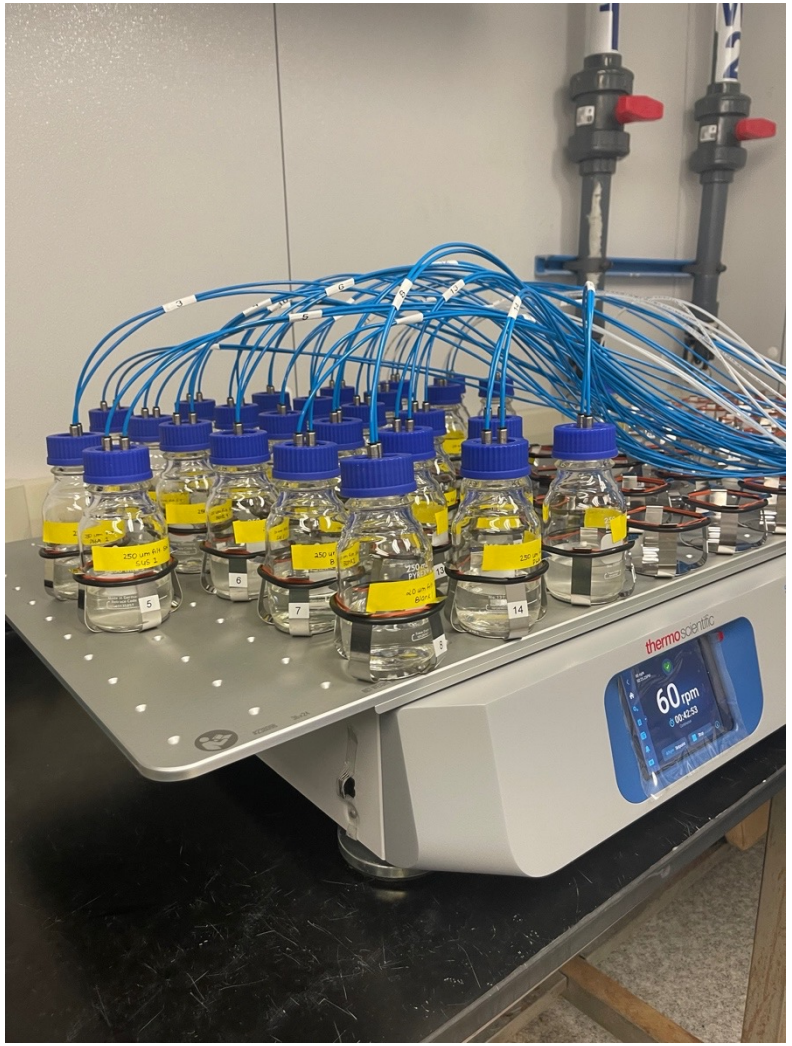
Host computer for fully automated sampling

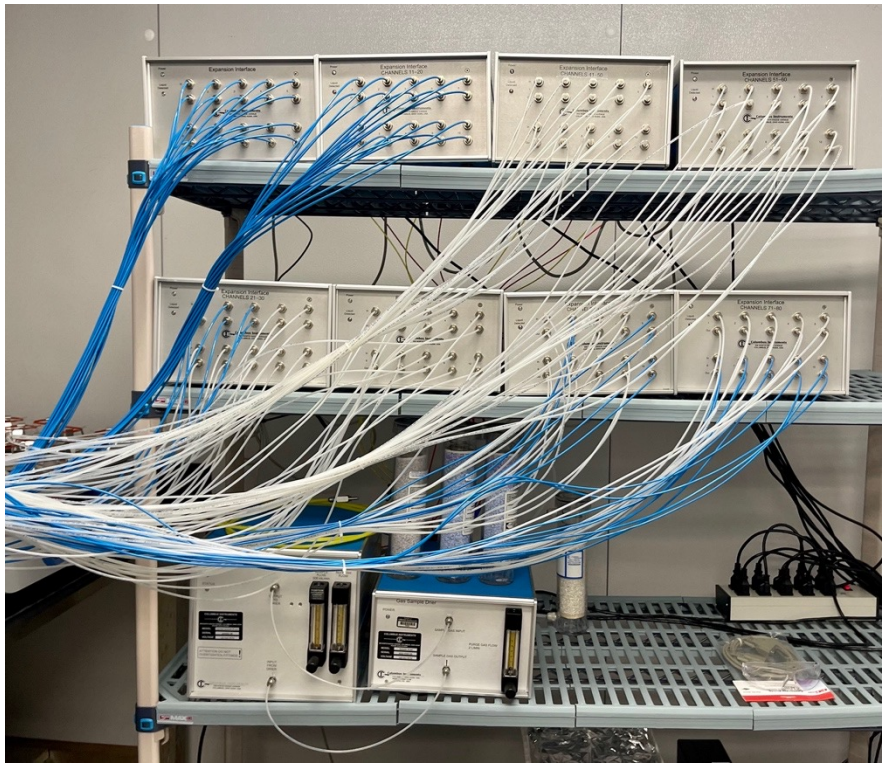
Sample drier: Remove water vapor from sample gas prior to concentration measurement

Sample pump: maintains constant flow rate (0.5 L/min) and pressure to prevent errors caused by barometric pressure changes

Micro-Oxymax 80 Channel System (ASTM D6691 & D5988)

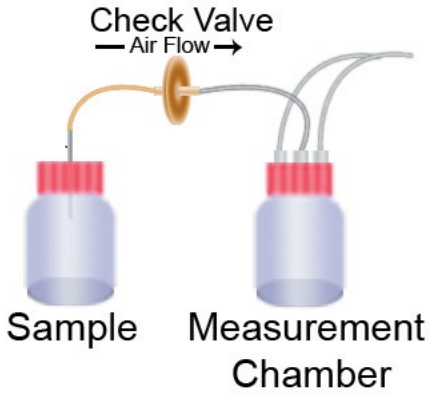
- Closed-loop system
- Aerobic
- Temperature and light controlled environmental control room set to 30°C
- 0-3% CO_2 Non-dispersive infrared detector
- Two orbital shakers w/ large platforms





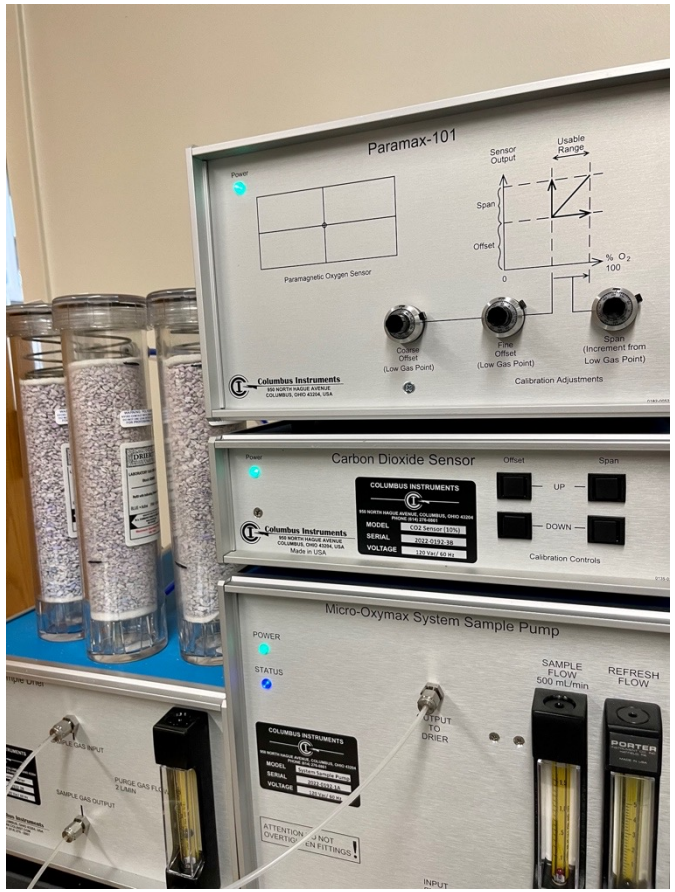
Micro-Oxymax 80 Channel System (ASTM D5511 & D5210)

- Two vessel closed-loop system
- Anaerobic
- Temperature and light controlled incubator set to 52 ℃
- 0-3% CO_2 and 0-5% CH_4 Non-dispersive infrared detector



Micro-Oxymax 40 Channel System (ASTM D5338)

- Open flow system (highly active samples)
- Aerobic
- Temperature and light controlled incubator set to 58 ℃
- 0-10% CO_2 Non-dispersive infrared detector
- 0-100% O_2 Paramagnetic sensor
- Condensing air driers
- 3.5 L wide-mouth reactor vessels



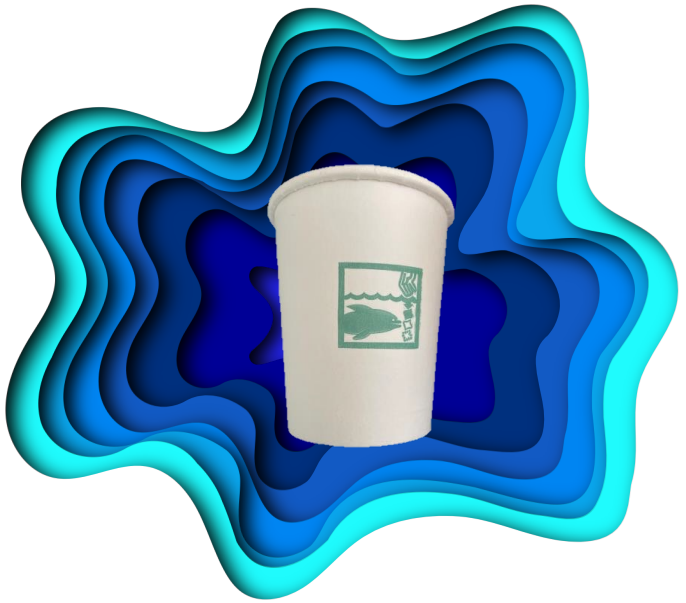
- Tier I – Standard methods in laboratory
  - Respirometry methods
  - ASTM D6691
    - Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum
- Tier II – Confirmatory in Marine Environment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

ASTM D6691 Standard Method

Environmental Conditions

ASTM standard method prescribed parameters

Carbon content (%C) of experimental and control substrates



Biochemical transformation during the aerobic incubation:

$C + O_2 \rightarrow CO_2$

Each mmole (12 mg) of organic carbon from the experimental/control substrate can be converted into 1 mmol of gaseous CO_2

<u>Total carbon in the experimental/control substrate:</u>

$$C_i\ (mmoles\ substrate) = \left(mg\ C\ (substrate) \times \frac{\%\ C\ (substrate)}{100}\right) \times \left(\frac{1\ mmoles}{12\ mg}\ C\right)$$

where:
% C (carbon content) is determined through elemental analysis (Coastal Systems Program).

ASTM D6691 Standard Method

Environmental Conditions

Adhering to ASTM standard method prescribed parameters and

- **Natural Seawater (pre- & post-incubation measurements)**
- Temperature
- pH
- Particulate organic carbon
- Macronutrients (required for bacterial activity)
  - Nitrogen
    - Particulate
    - Dissolved (ammonium, nitrate+nitrite)
  - Dissolved phosphorus (ortho-phosphate)
- Salinity

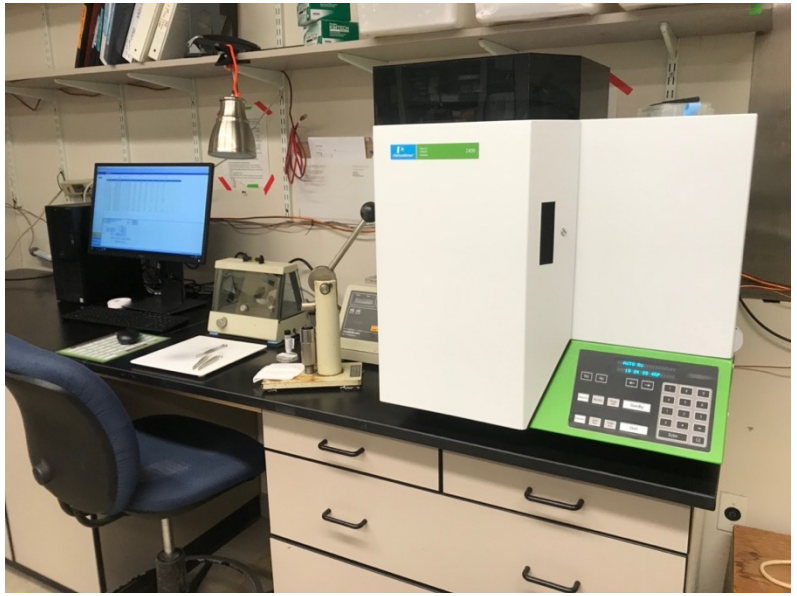
Coastal Systems Program Instrumentation

- Perkin Elmer Elemental Analyzer for CN analysis
- pH meter/titrator
- Conductivity meter
- Seawater filtration setups and drying ovens for POCN and Chla
- Spectrometry/autoanalyzer for full suite of N species and phosphorus

Ammonium & orthophosphate measured via optical density

SMAST

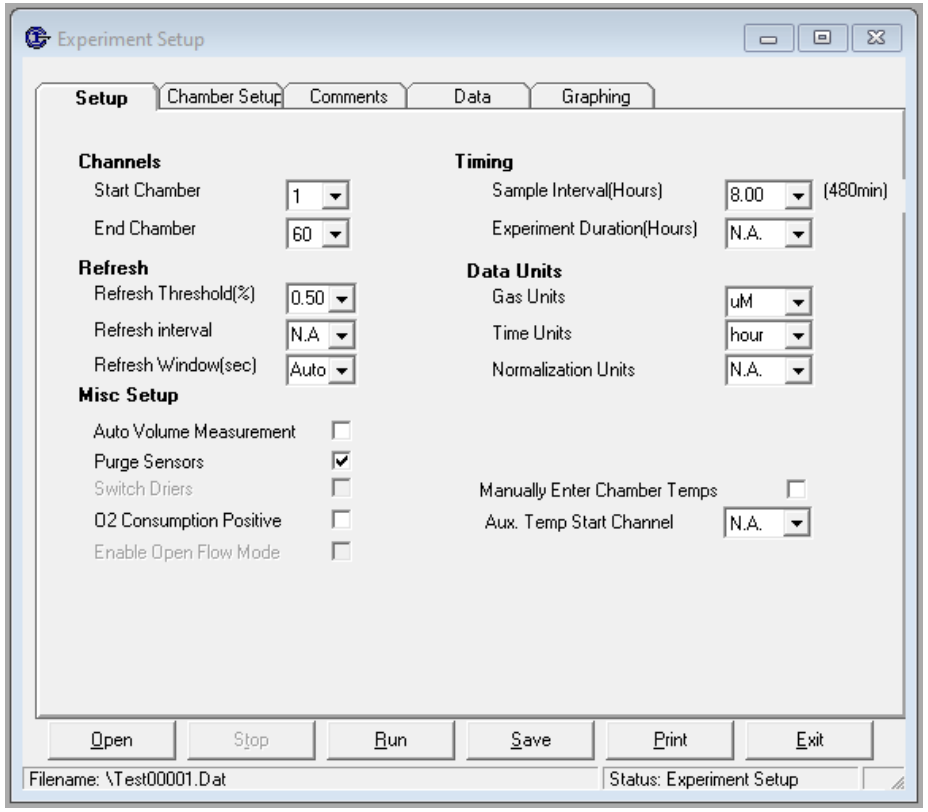
- Seawater lab for raw seawater collection
- -32C freezer and 4C cold room for sample storage
- Autoclave/deionized water

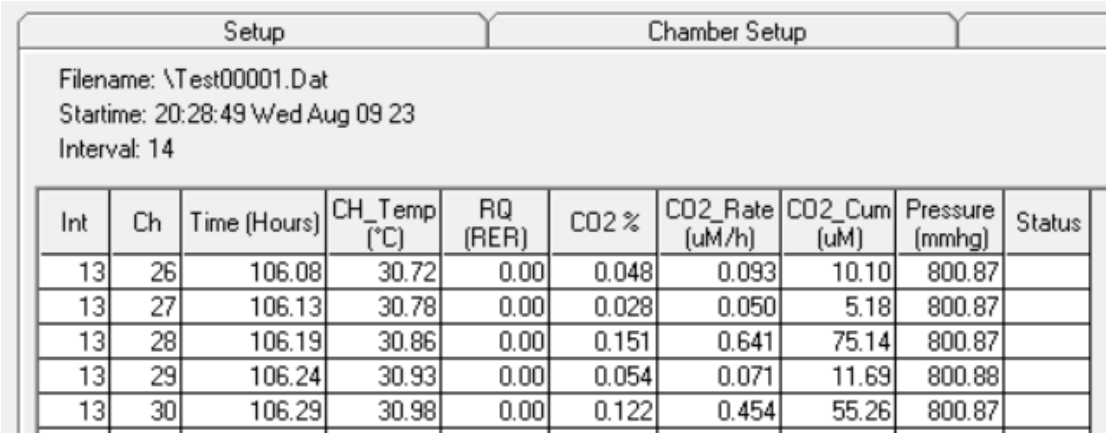


ASTM D6691 Standard Method

Biodegradability Lab First Year Milestones

- Setup and installation of Micro-Oxymax respirometers
- Preliminary Tests of seawater blanks and positive controls
- Standard operating procedure in accordance with ASTM D6691
  - Experiment Parameters
    - 250 ml vessels
    - 75 ml seawater per vessel
    - 20 mg experimental/control substrate
  - Micro-Oxymax & Instrument Parameters
    - 60 rpm shaker speed
    - 8 hr sampling interval





Int	Ch	Time (Hours)	CH_Temp (°C)	RQ (RER)	CO2 %	CO2_Rate (uM/h)	CO2_Cum (uM)	Pressure (mmhg)	Status
13	26	106.08	30.72	0.00	0.048	0.093	10.10	800.87	
13	27	106.13	30.78	0.00	0.028	0.050	5.18	800.87	
13	28	106.19	30.86	0.00	0.151	0.641	75.14	800.87	
13	29	106.24	30.93	0.00	0.054	0.071	11.69	800.88	
13	30	106.29	30.98	0.00	0.122	0.454	55.26	800.87	

ASTM D6691 Standard Method

Variables to Consider

- Natural seawater vs defined culture
  - We never explored the defined culture because we have a readily available water source (SMAST seawater lab)
- Natural seawater
  - Seasonality
    - Water quality constituents (e.g., particulate organic matter)
    - Microbial community composition
  - Long term water quality monitoring at SMAST Pier

ASTM D6691 Standard Method

- Variables to consider (continued)
  - Seawater handling
    - Collection
      - Raw seawater collection from the SMAST seawater lab
      - By Boat or pier using Niskin water sampler or geopump
        - Volume needed, depth/current
    - Storage
      - Temperature (ambient or 30°C)
      - Duration (same-day vs. weeks)
      - Aeration

ASTM D6691 Standard Method

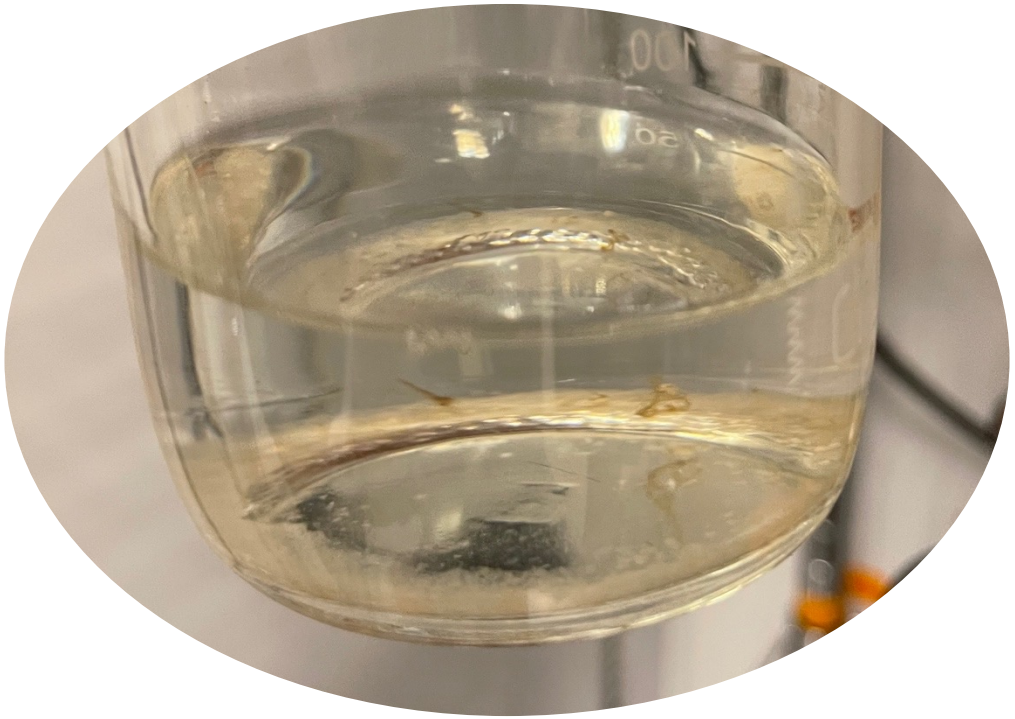
Variables to Consider (continued)

- Positive control (cellulose, chitin, Kraft paper)
  - Sigmacell cellulose, Type 101
    - Dispensed as a suspension
- Polymer form (fiber or film vs microcrystalline cellulose)
  - Experimental substrate should be in the same form as controls
    - Cryomill, Retsch Mixer Mill MM400 with LN_2

ASTM D6691 Standard Method

Variables to consider (continued)

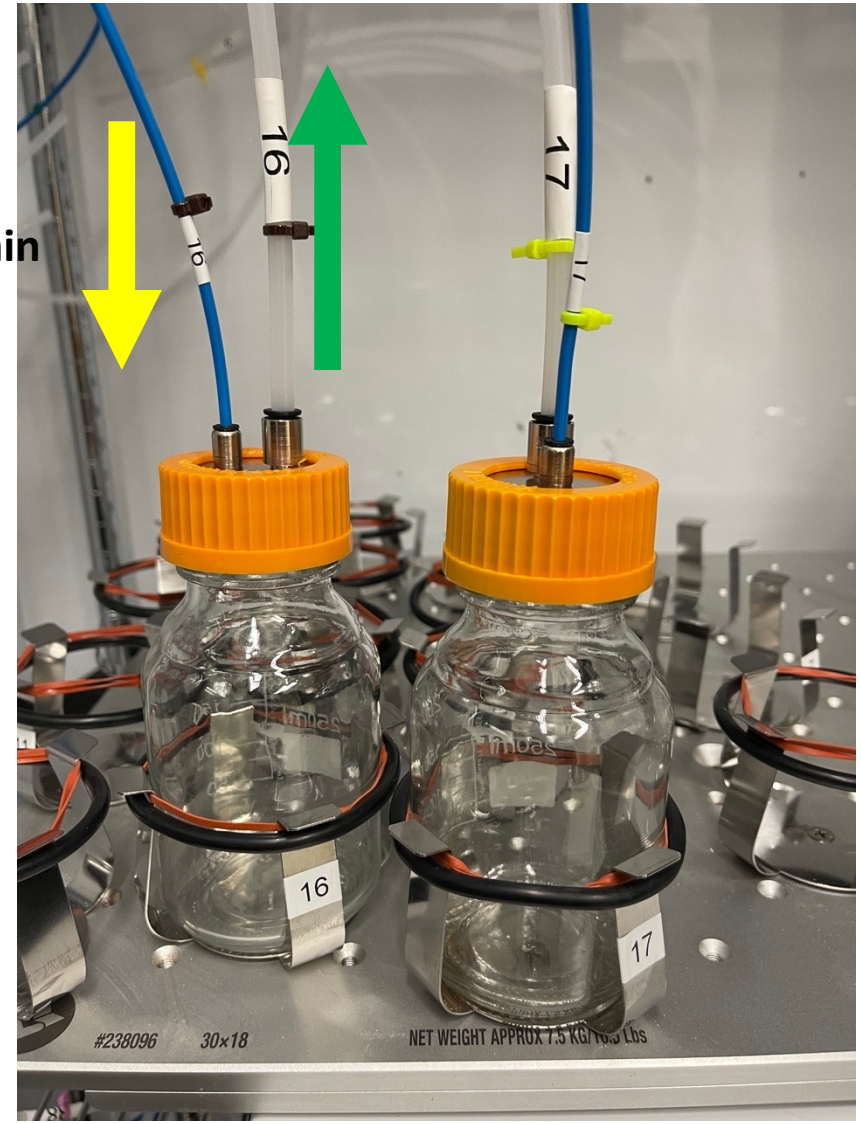
- Cleaning reactor vessels
  - Scrub/rinse, acid bath, and autoclave



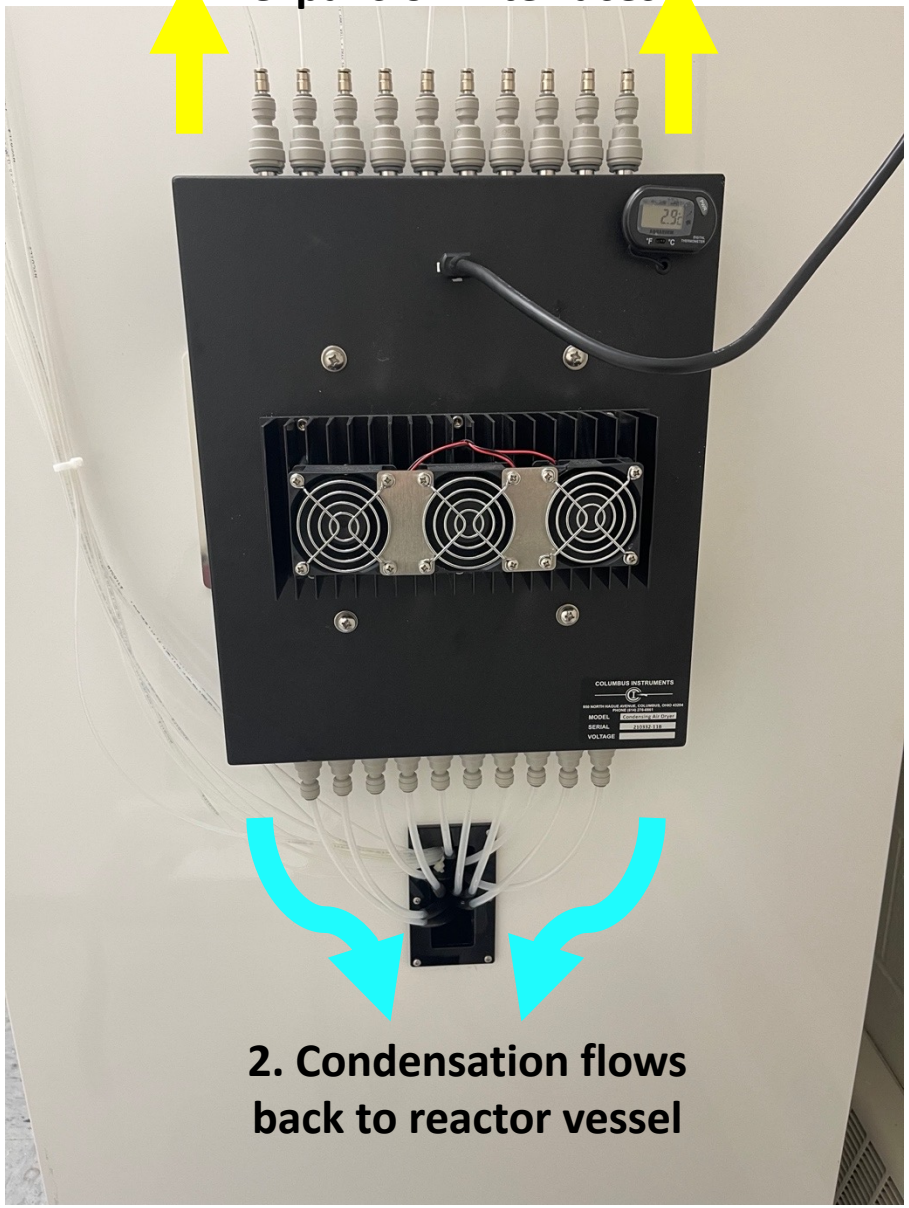
Biofilm formation

1. Sample gas + water vapor returned to vessel (0.5 L/min flow rate)

4. Sample gas returned to vessel (0.5 L/min flow rate)



3. Sample gas flows to expansion interfaces



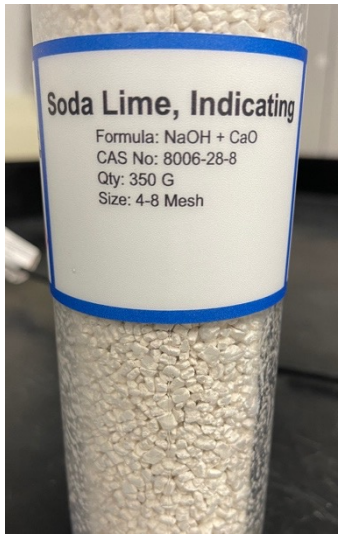
2. Condensation flows back to reactor vessel

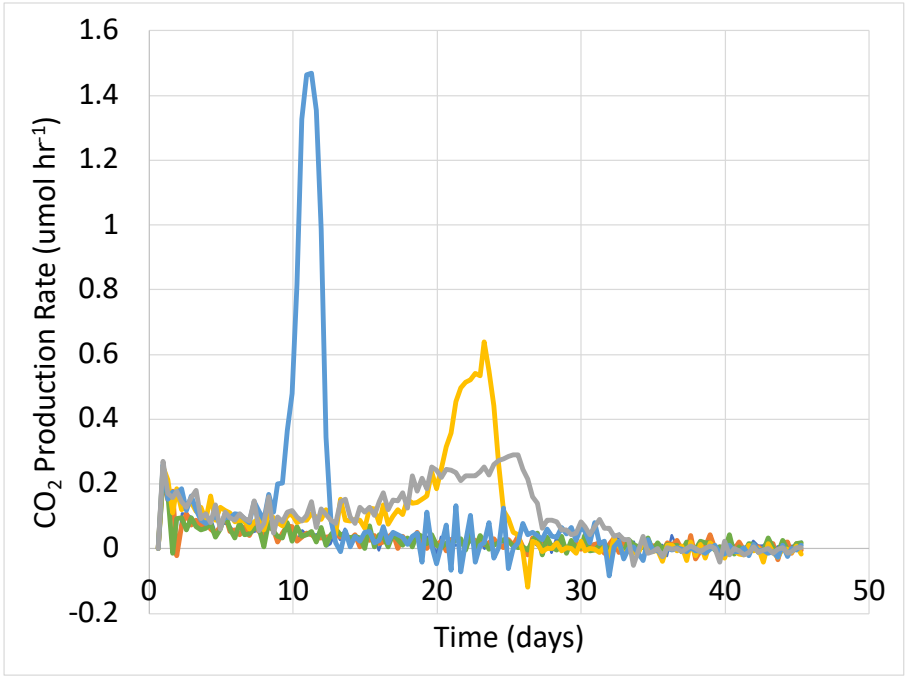
INCORRECT SLOPE

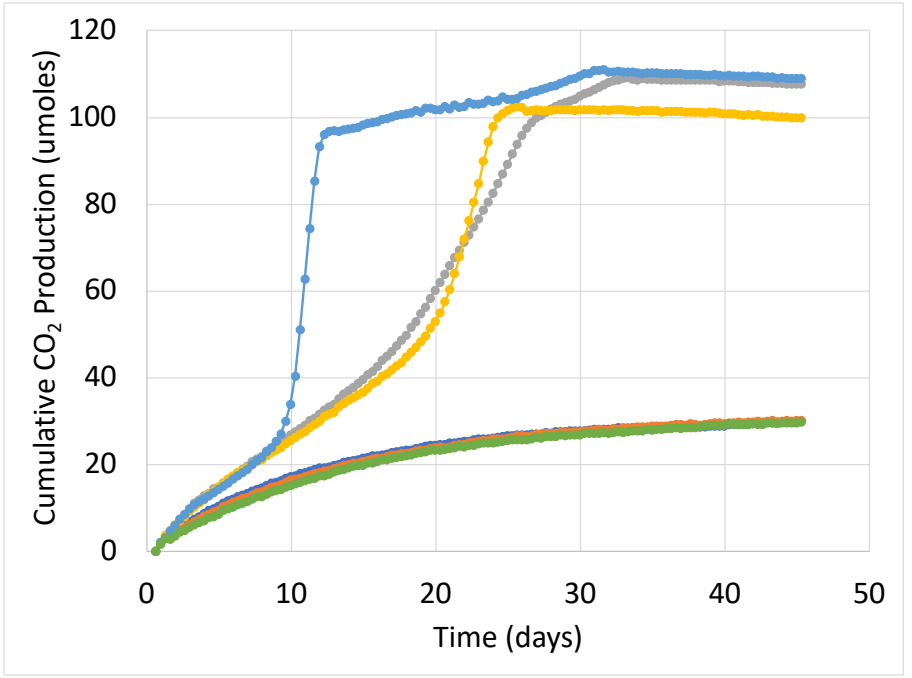
CORRECT SLOPE

UNEXPECTED ISSUES

- Standard method not so standard. Differences between groups running the ASTM D6691 Standard Method
  - Shaker speed (175 rpm vs 60 rpm)
- Negative cumulative rates in seawater blanks.
  - Scrub CO_2 from refresh and sensor purge air with soda lime column
- Variability in seawater blank replicates







Next Steps

- Complete validation experiments
  - 70% biodegradation of the positive control
  - Consistent blank and positive control replicates (std dev < 20% of the mean, ideally < 5%)

% Biodegradation =

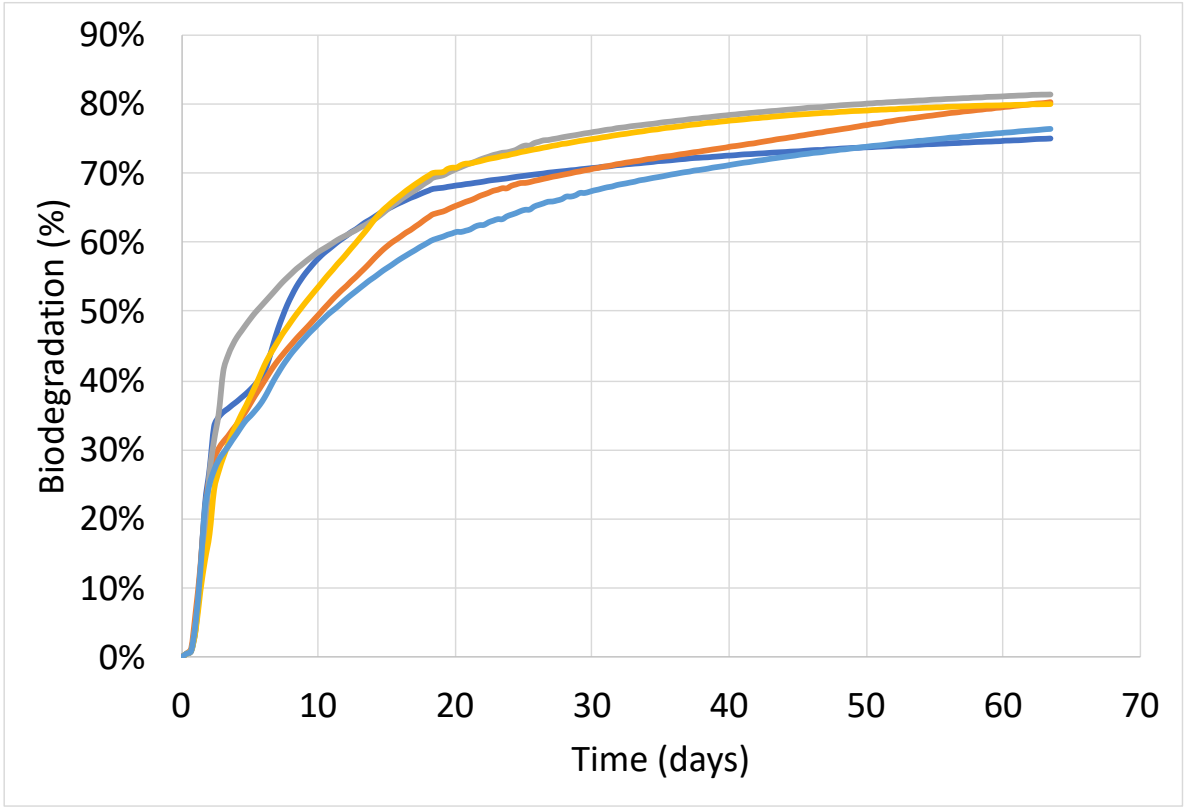
$$\frac{mean\ C_g\ (substrate)\ -\ mean\ C_g\ (blank)}{C_i\ (substrate)} \times 100$$

where:

C_g = amount of gaseous carbon produced from the experimental/control substrate and culture blank, µmoles, and

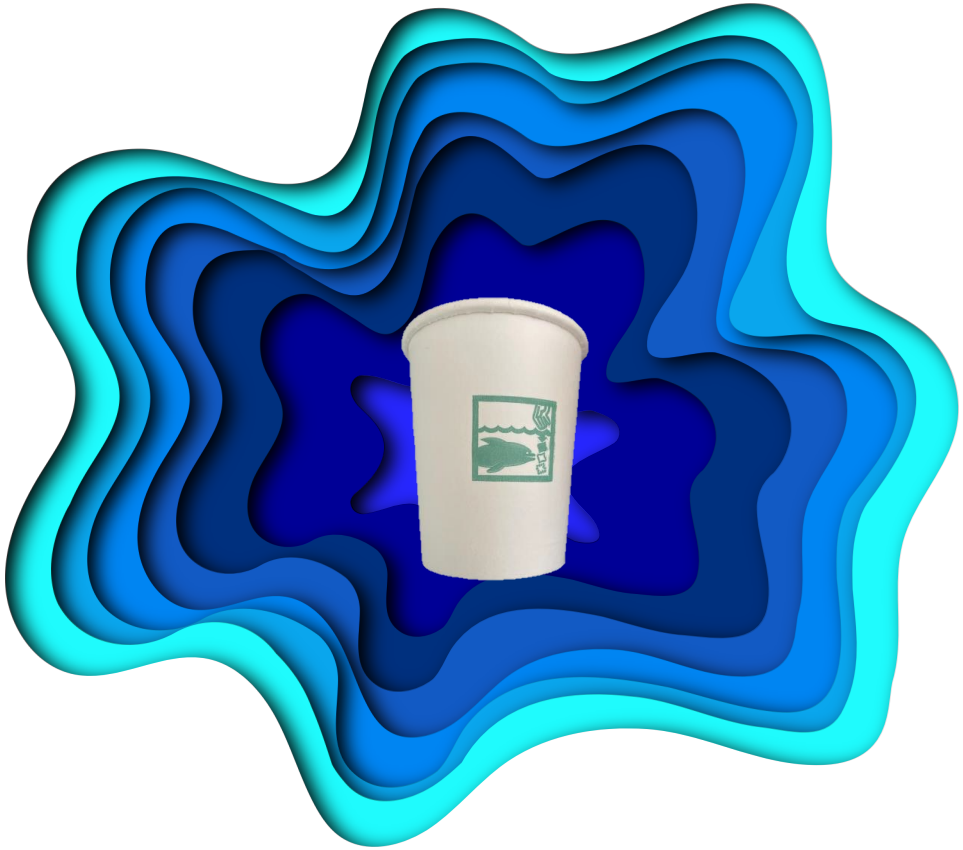
C_i = amount of carbon in experimental substrate added, µmoles.

$$\frac{544.26\ umoles - 27.50\ umoles}{656.2\ umoles} \times 100 = 78.6\%$$



Dr. Jo Ann Ratto Ross

Professor Linda A. Amaral-Zettler



Coastal Systems Program
Eden Research Labs

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THANK YOU

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

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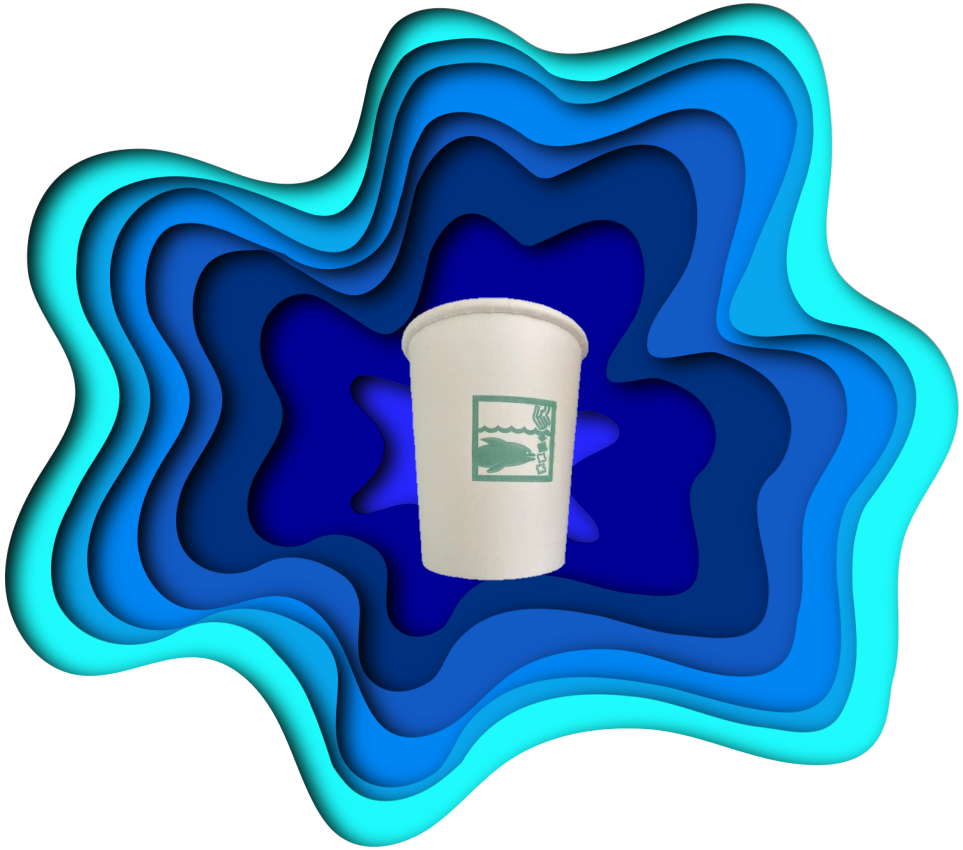

Questions?

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