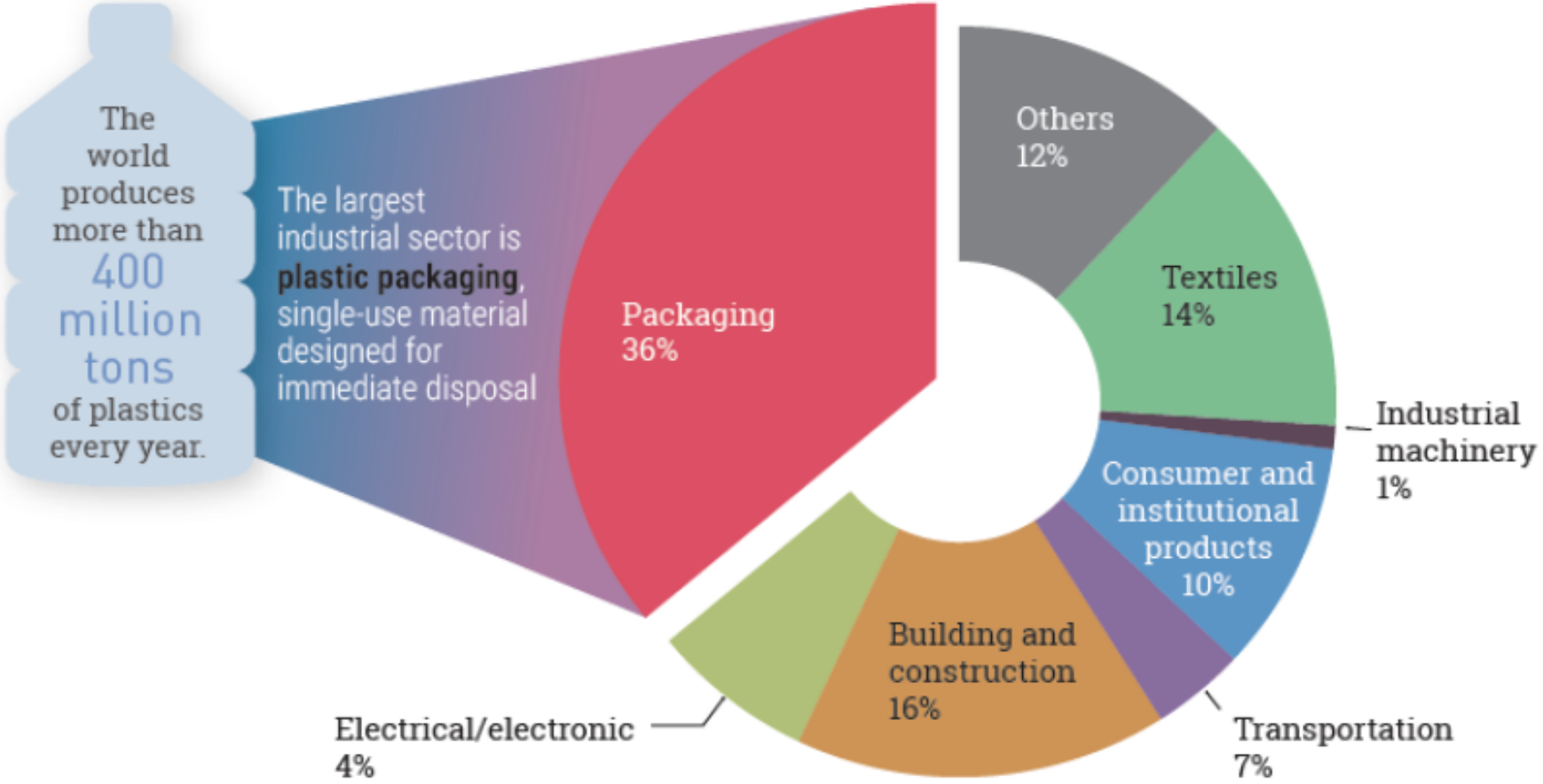
Biosourcing for Microbially Driven Polyethylene Degradation

K. Kucharzyk, R. W. Murdoch, J. Lilly, S. Higgins, M. Evans, E. Beasley, C. DeSanti
Battelle Memorial Institute

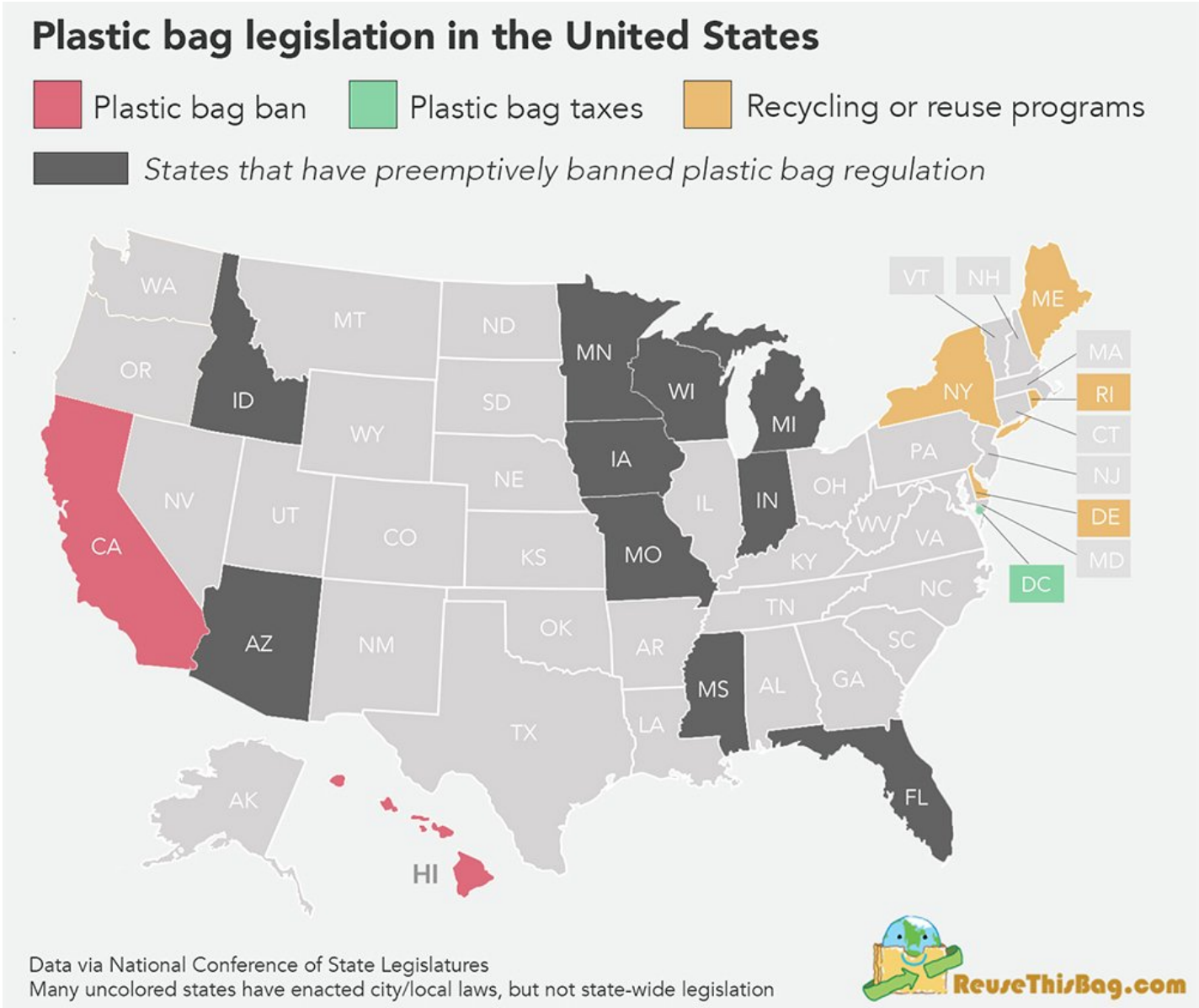
Plastics in the Environment

- Millions of tons of plastic, worth billions of dollars, ends up in landfills, is burned, or leaked into the environment
- This results in wasted material and energy resources and negative environmental impact



Regulation is Coming

- Some laws in place - focused on single use plastics
  - Eight states have banned single-use plastic bags. California, Hawaii, New York, Connecticut, Delaware, Maine, Oregon and Vermont.
  - Vermont has adopted the most comprehensive plastics ban in the US- regulating bags, straws, drink stirrers and foam food packaging.
  - Maine has a ban plastic foam containers beginning 2021, followed by Maryland.
  - 300+ municipalities across the US have single-use plastic bag ban.

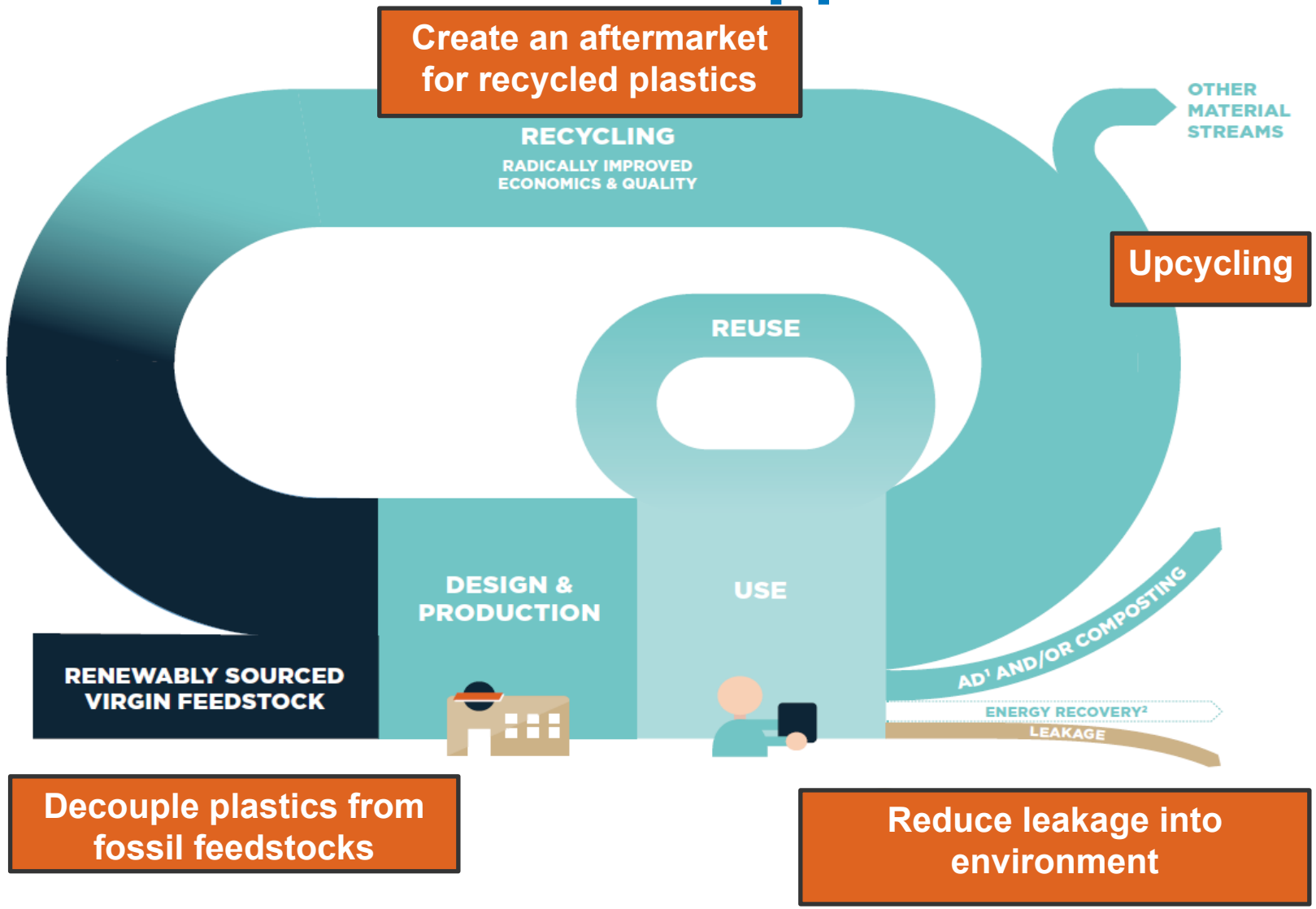


Technical Solutions are Needed to Support Plastics Circularity

Energy:
≥ 50% energy savings relative to virgin material production

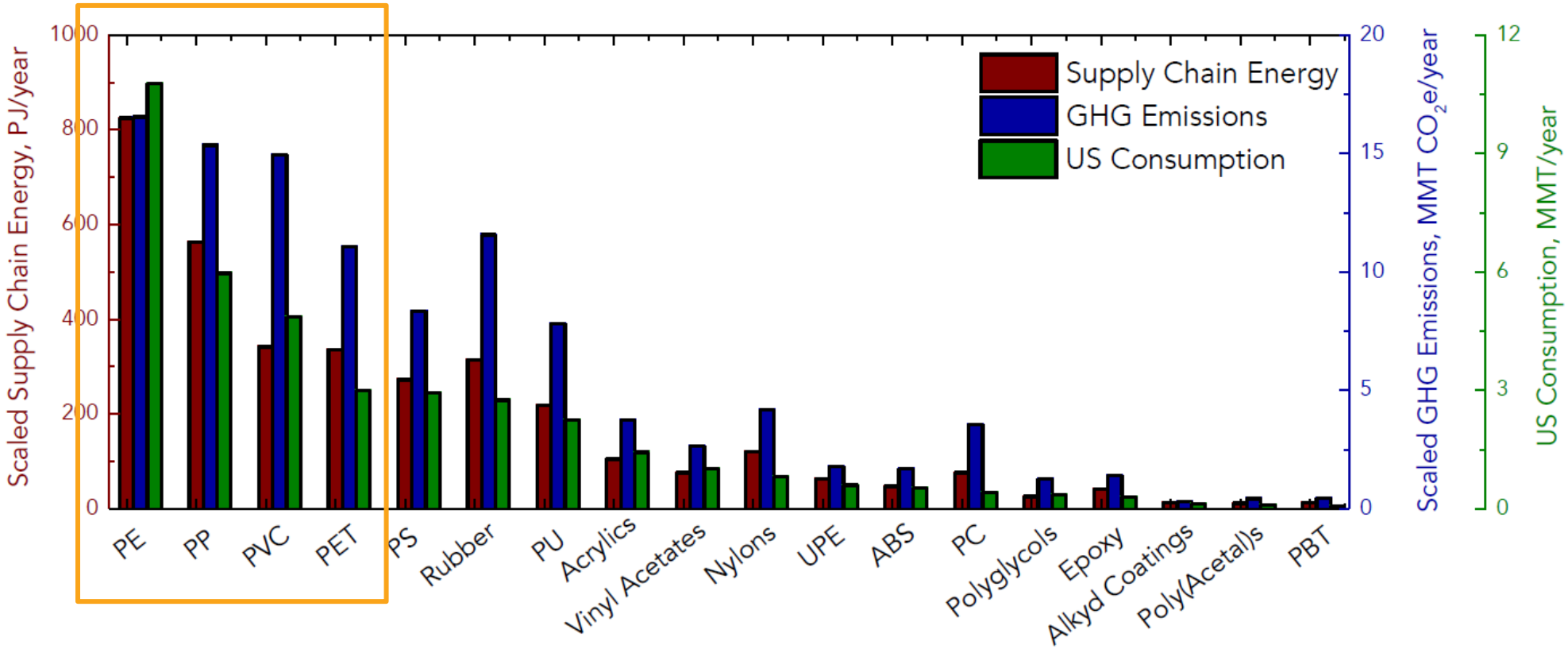
Carbon:
≥75% carbon utilization from waste plastics

Economics:
≥ 2x economic incentive above price of reclaimed materials



BATTELLE

Annual US Consumption per Target Polymer



- Variety of polymers used globally, with high energy consumption and GHG emission during production
- Battelle targets selected based on technology gaps, annual production, potential savings through alternative processes, and market attractiveness

LDPE vs HDPE Structure

LDPE

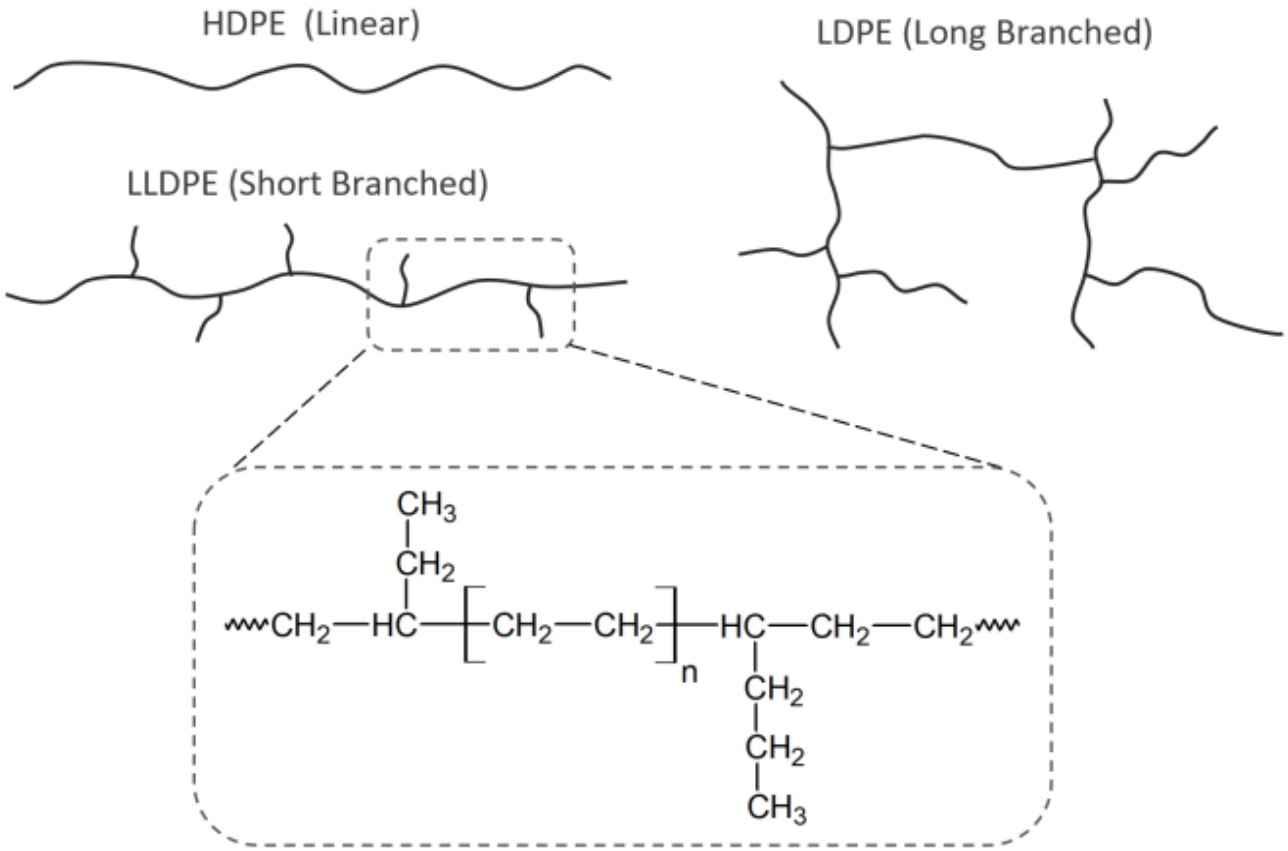
Low-Density Polyethylene

- Bin bags
- Packaging

HDPE

High-Density Polyethylene

- Milk bottles
- Carrier bags

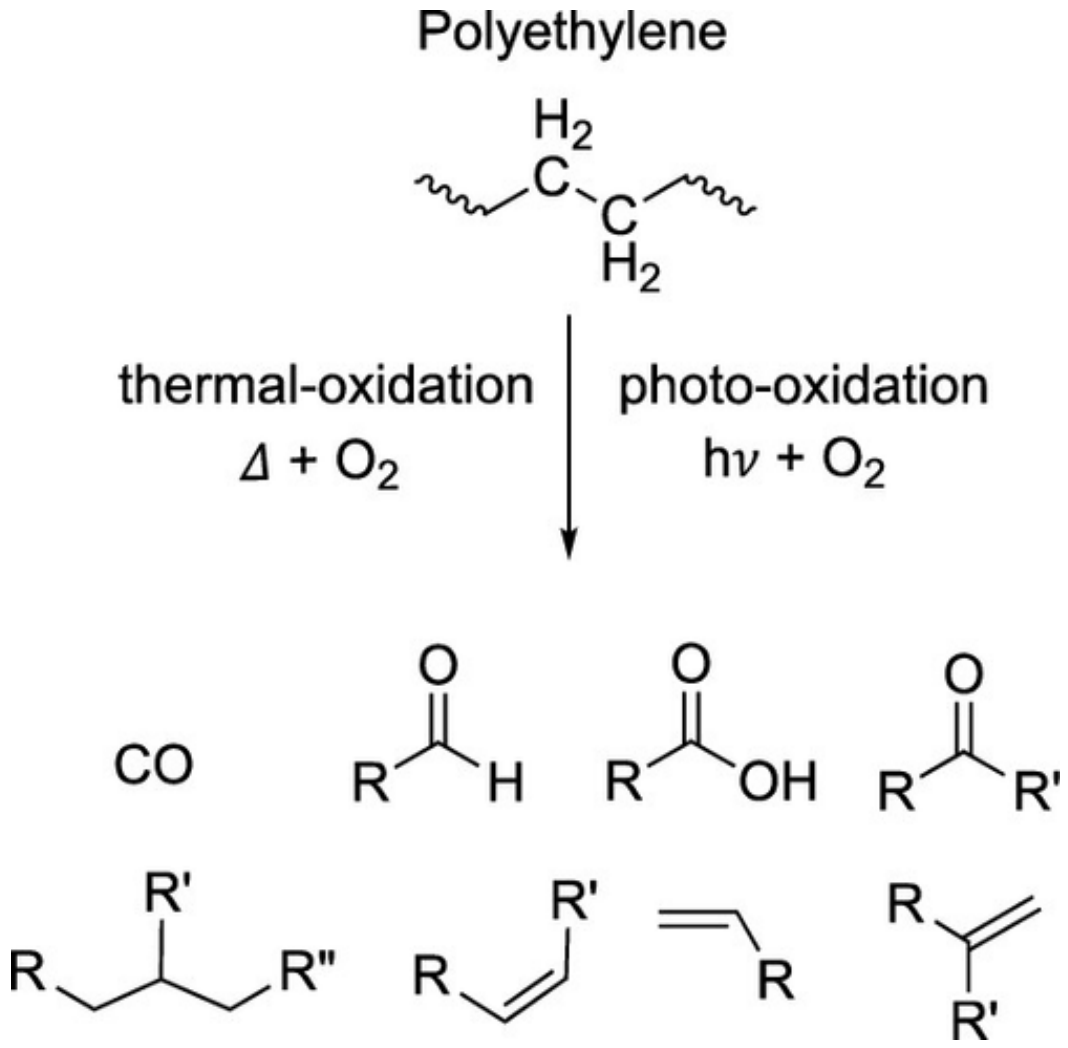


https://polymerdatabase.com/polymer%20classes/Polyolefin%20type.html

BATTELLE

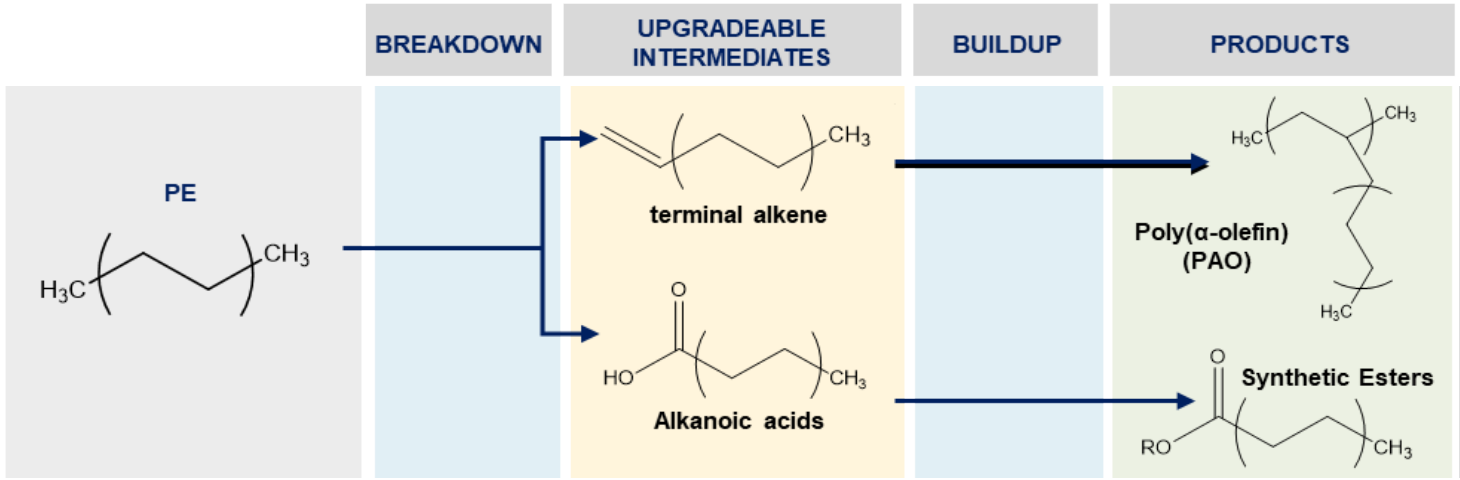
Known Chemical Degradation Routes

- The backbone chains constructed from C−C single bonds which do not undergo hydrolysis, and resist photo-oxidative degradation.
- PE may contain unsaturated bonds in the main chain or at the chain ends (vinyl groups in HDPE and vinylidenes in LDPE).
- These sites are readily oxidized by O_3 , NOx, or other tropospheric radicals, often to highly unstable hydroperoxides, converted to more stable UV-absorbing carbonyl groups.



Biodegradation is Possible but Slow

- Several microbial and fungal candidates known to be associated with potential for degradation of C-C bonds in HDPE



Fungal strains associated with polyethylene biodegradation.

Genus	Species	References
Aspergillus	*niger*	[91,20,119]
	versicolor	[149,69]
	flavus	[121,63]
Cladosporium	*cladosporioides*	[63,116]
Chaetomium	sp.	[152]
Fusarium	*redolens*	[70,69,23]
Glioclodium	*virens*	[91]
Mucor	*circinelloides*	[121]
Penicillum	*simplicissimum*	[61]
	pinophilum	[91,20]
	frequentans	[100]
Phanerochaete	*chrysosporium*	[91,71,144]
Verticillium	*lecanii*	[69]

Ma, J., Xu, M., Wu, J., Yang, G., Zhang, X., Song, C., ... & Wang, Y. (2023). Effects of variable-sized polyethylene microplastics on soil chemical properties and functions and microbial communities in purple soil. Science of The Total Environment, 161642.

Sourcing Microbial Candidates from Environments

DoD samples from hydrocarbon contaminated sites

- DoD sites impacted with hydrocarbon contamination

NOAA oceanic Mariana Trench samples from plastic contaminated sediments

- 9 samples from various locations in the western Pacific Ocean

Plastic degrading fungi

- *Streptomyces noursei*; ATCC 11455, *Streptomyces eurocidicum*, *Streptoverticillium thioluteum*, *Streptomyces achromogenes*, *Ganoderma lucidum*

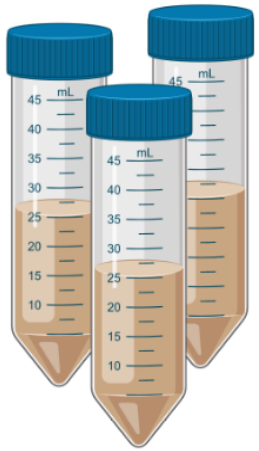
Microbial cultures of literature reported plastic degraders

- *Rhodococcus opacus* PD630, *Rhodococcus ruber*, *Rhodococcus jostii*, *Rhodococcus wratislavensi*

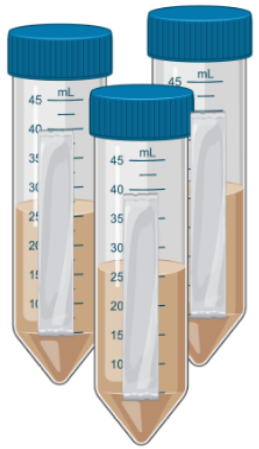
Sourcing Microbial Candidates from Environments

- Battelle collected samples from the Solid Waste Authority of Central Ohio (SWACO) in Grove City, OH.
- The samples comprised loose soil, packed soil, and plastic samples from an approximately 10-year-old land-filled waste slope.
- SWACO personnel assisted us by opening sampling holes with a back-hoe to allow access to buried soil/plastic mixtures.
- The samples are being extracted and analyzed now.

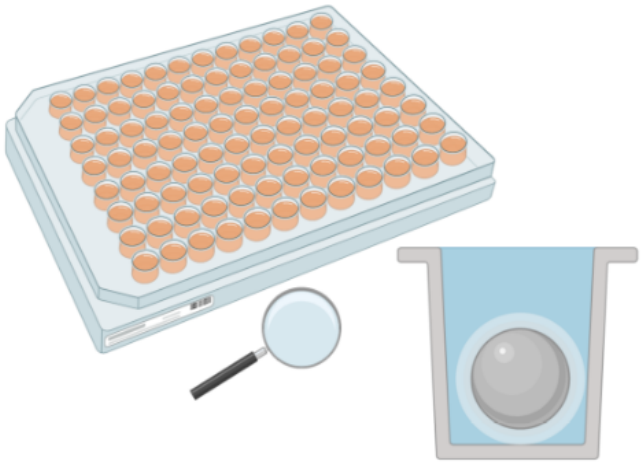
Enrichments and Experimental Setup



Flasks with powdered LDPE + microorganisms



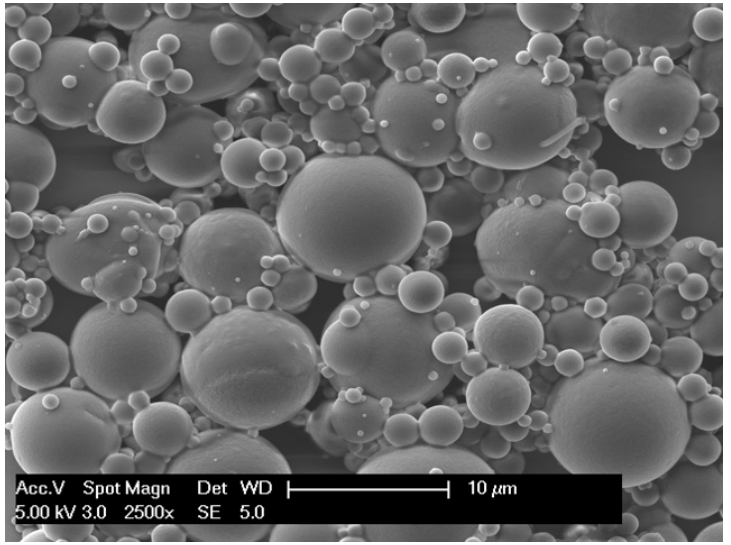
Flasks with LDPE film + microorganisms



96-well plates with 1180-1140 um PE beads + microorganisms

PE enrichment setup. Experimental vessels are set up in duplicates, kept incubated in aerobic conditions with agitation at 29 C.

Crushed LDPE Goodfellow feedstock and LDPE film



Microspheres - Polyethylene spherical particles 1 micron to 10 micron (1 µm to 10 µm) in diameter – Unpigmented. Three ranges of sizes are explored in the program: 32-38 µm, 800-1000 µm and 1180-1400 µm.

BATTELLE

PE Extraction Methods

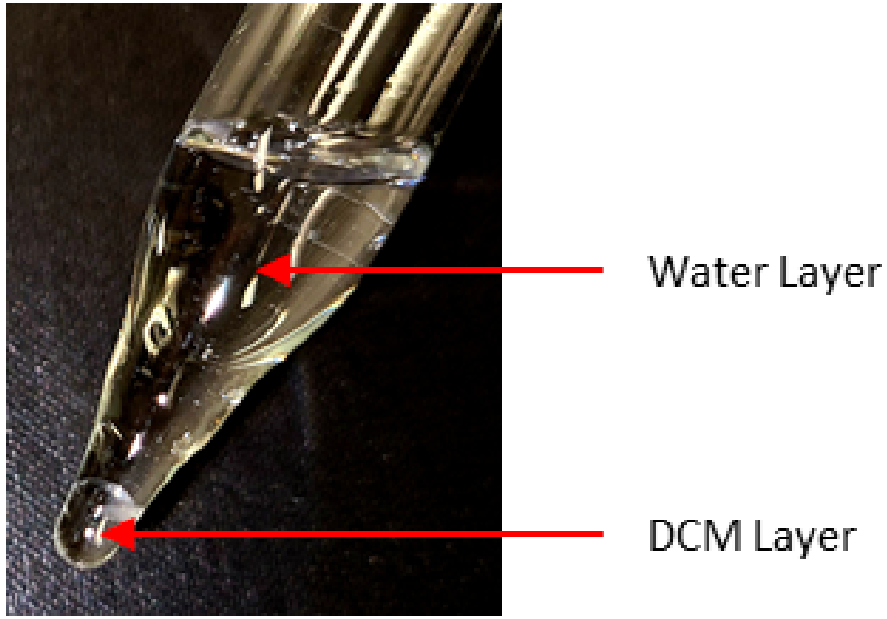
PE Analysis
Completed development on an **extraction method for** detection of PE biodegradation products in small (1-2 mL) sample volumes. The method allows to pre-concentrate and clean products such as dodecanol with minimal volume of starting sample and number of steps through the process for increased high throughput.

Sample Extraction Testing

- 1 mL of reagent water spiked with 1-decene and 1-decanol (2 µg/mL final concentration)
- Liquid-liquid microextraction with methylene chloride
- Analyze by GC/MS
- Average Recoveries (n=2)

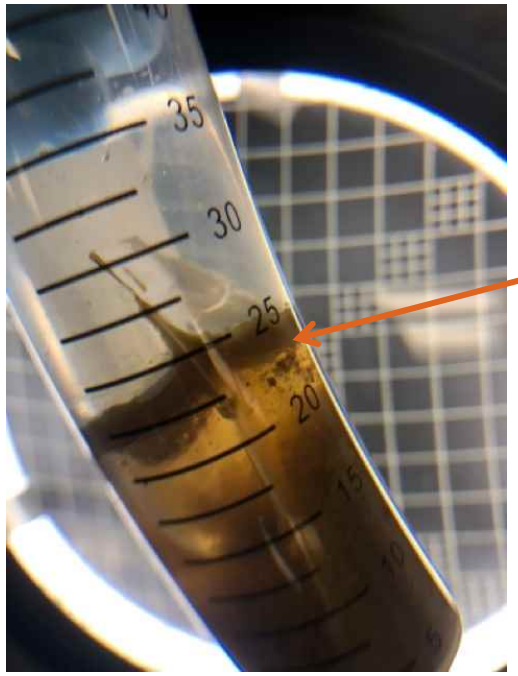
Next Steps

- Add decanoic acid to test mix
- Repeat extractions with 5 mL of sample
- Reduce methylene chloride volume to increase the enrichment factor



Qualitative Observations

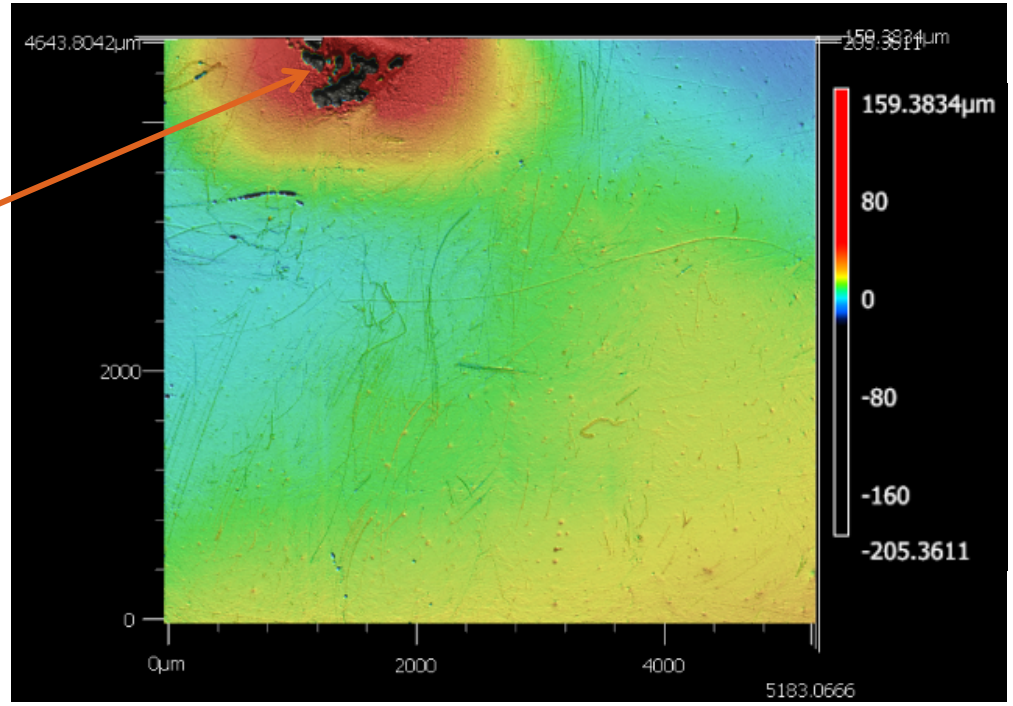
20-day biosourcing experiment and made qualitative observations of all samples. Further PE analysis ongoing.



Thick biofilm colonizing LDPE film

Most PE films show formation of biofilms in NOAA sourced samples and fungal samples

Mechanical defect in the LDPE film



Keyence VK-X260 3D laser scanning confocal microscope analysis to monitor change in surface roughness consistent with microbial attack in the films

PE Analysis in 1st Round of Environmental Enrichments

Additional confirmatory analyses show **that five out** of 30 environmental enrichments grown on LDPE **produce acids during degradation of LDPE.**

Sample Name	Octanoic acid	Nonanoic acid	Decanoic acid	Dodecanoic (Lauric) acid	Tetradecanoic (Myristic) Acid	n-Hexadecanoic (Palmitic) Acid	Growth increase over time
	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	
Michigan	0.58	3.02	0.00	0.00	0.00	0.00	yes
NOAA 010	1.55	4.73	6.90	<10	22.50	>10	yes
S. thioluteum (YM)	8.00	7.50	0.00	0.00	50.57	>10	yes
S. noursei (YM)	0.70	1.00	0.00	0.00	6.32	0.00	yes
Landfill Leachate	1.44	0.00	29.83	0.00	0.00	0.54	yes

- ***Tested solvents***: hexane, chloroform, isopropanol, ethanol show varied recoveries with chloroform performing with close to 100% recovery of surrogates.
- Application of an extraction method with better acid recovery allows to confirm identification of samples with the highest potential for PE degradation.

PE Analysis in 2st Round of Environmental Enrichments and SWACO Samples

2nd round of re-inoculated PE-degrading samples show higher concentrations and bigger variety of produced acids. No production of acids in SWACO samples after 21-days of incubation.

Sample Name	1-Nonene	1-Decene	1-Octanol	1-Dodecene	Octanoic acid	Nonanoic acid	Decanoic acid	1-Dodecanol	Dodecanoic (Lauric) acid	Tetradecanoic (Myristic) Acid
NOAA 116 0X	0.59	0.68	0.86	0.59	0.00	0.00	11.12	0.22	0.00	0.00
NOAA 116 QC	0.00	0.00	0.00	0.15	0.00	0.25	0.00	0.00	0.00	0.00
Land Leachate 0X	0.00	0.14	0.00	0.18	0.00	0.27	0.00	0.00	0.00	0.00
Land Leachate QC	0.27	0.24	0.00	0.33	0.00	0.18	0.00	0.00	0.00	0.00
S. thioluteum 0X	0.00	0.00	0.00	0.24	0.27	0.82	15.42	0.00	5.91	75.51
S. thioluteum QC	0.00	0.00	0.00	0.00	0.37	1.01	0.00	0.00	6.50	77.19
S. noursei 0X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. noursei QC	0.00	0.00	0.00	0.23	0.00	0.41	11.42	0.00	0.00	14.20

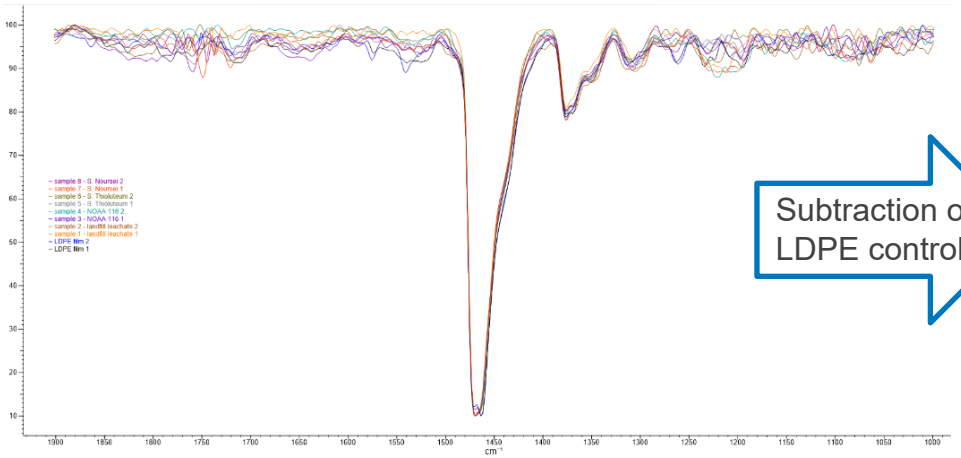
QC – normal LDPE control OX – pre-oxidized LDPE

- Battelle added oxidized PE material to the tested matrix, which generated broader spectrum of acids than the native feedstock/
- Previously down selected samples of the Phase 1 enrichments show faster and higher conversion of the feedstock and generation of acids.
- SWACO samples in some cases show change of color during the 21-day degradation experiments however, no acids were detected from the PE degradation yet. The incubations will be continued for another 20 days.

Environmental Enrichment Sample Analysis by FTIR-ATR

ATR scans were obtained of from cleaned LDPE films after incubation with downselected environmental enrichments. Subtraction of spectra from negative control reveals residual peaks associated with oxidation (C=O) at 1700-1800 cm^{-1}. This suggests microbial modification of the surface.

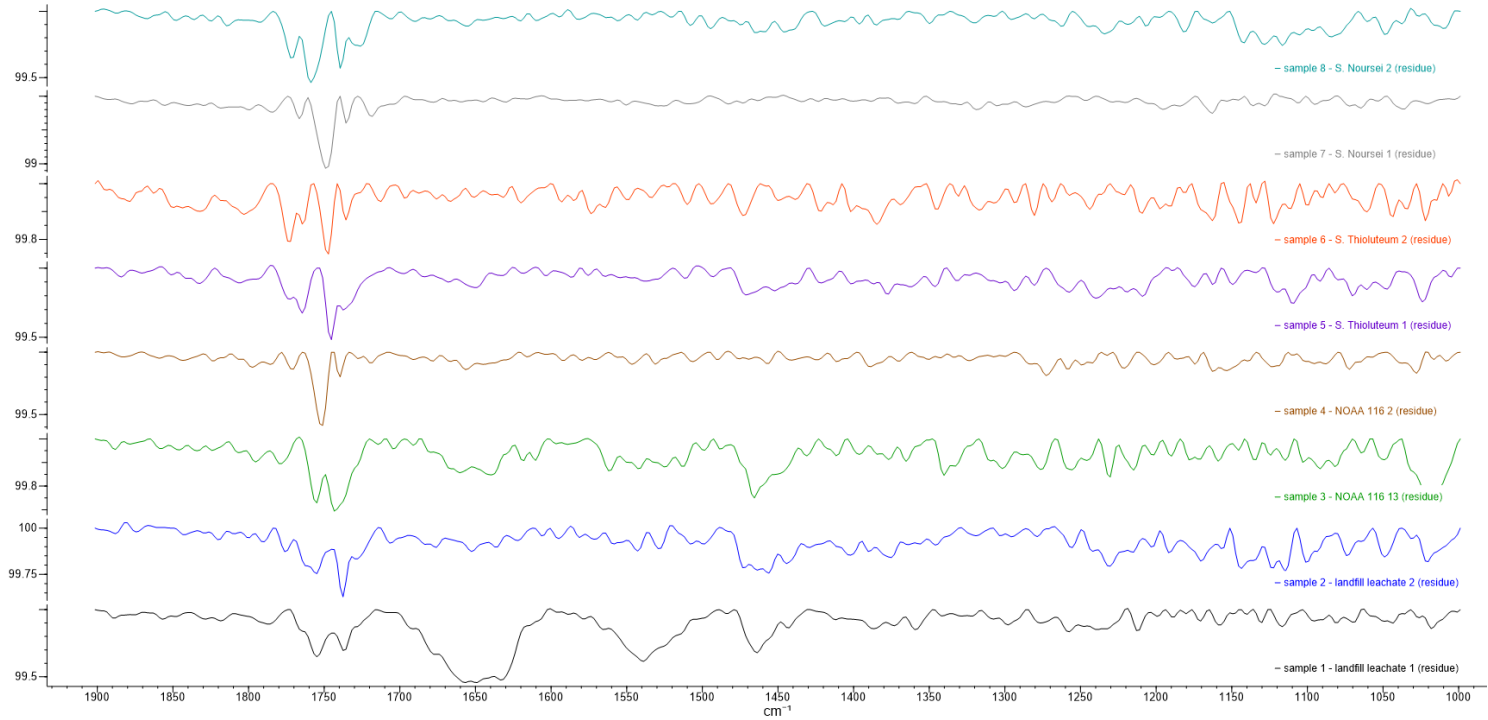
Raw ATR Scans



Subtraction of LDPE control

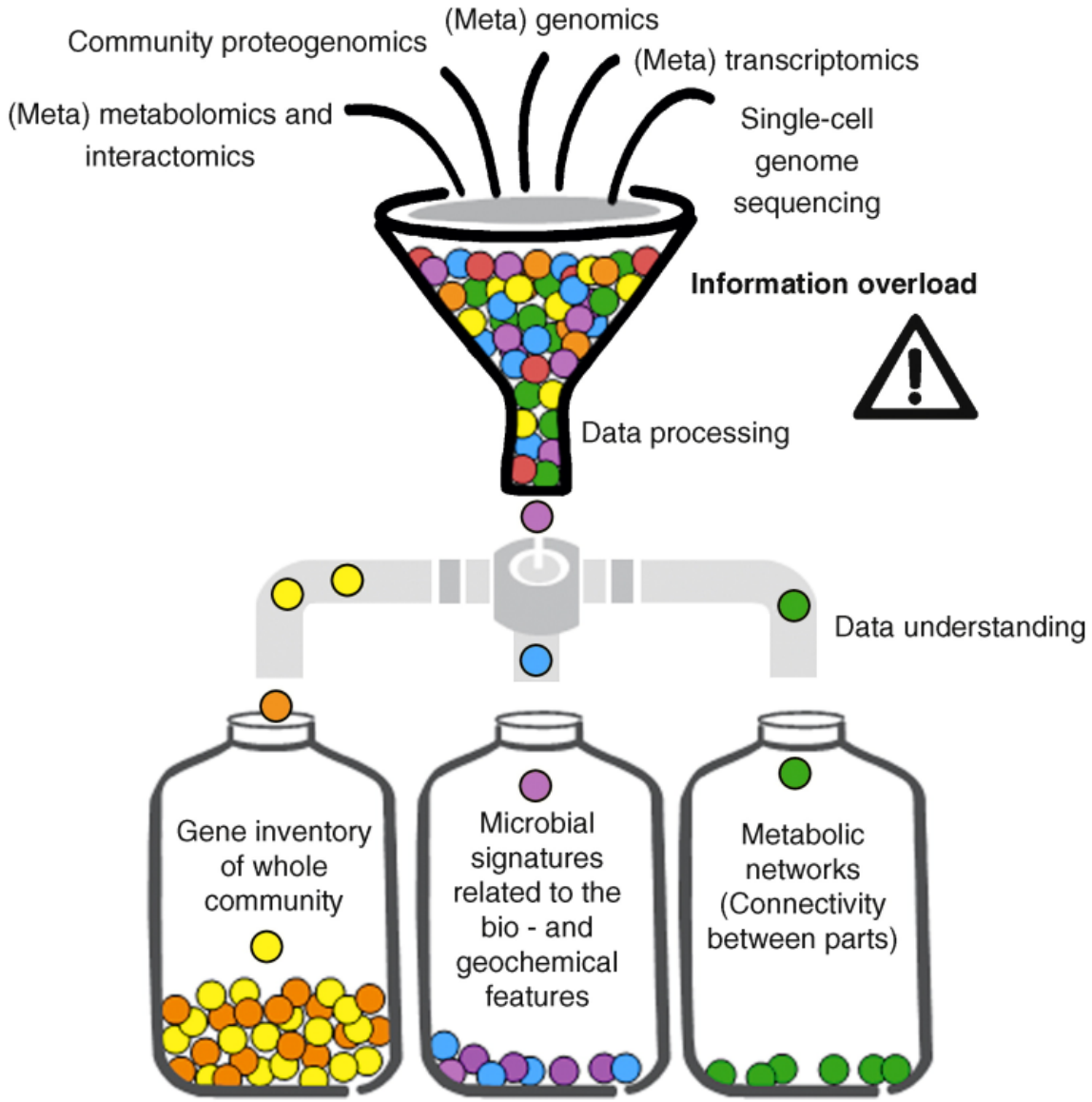
Working to increase scan resolution to reduce noise for more detailed analysis

Residual spectra of samples incubated with enrichments



Towards Increased PE Degradation and Molecular Level Understanding

- Microbial enrichments adapt to C source in time.
- The Phase 1 Enrichments degrade LDPE faster after re-inoculation of established cultures and dosing with fresh LDPE.
- Next steps are focused on omic analyses of enrichments and pure cultures to understand genetic and biochemical basis of PE degradation.



Acknowledgements

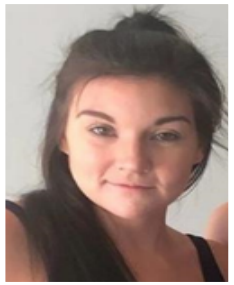

Chuck DeSanti


Robert Murdoch

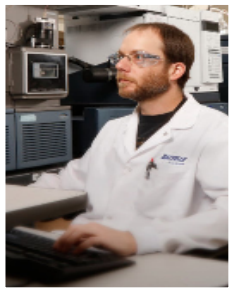

Ashley Frank


Steven Higgins


Colin Hinton

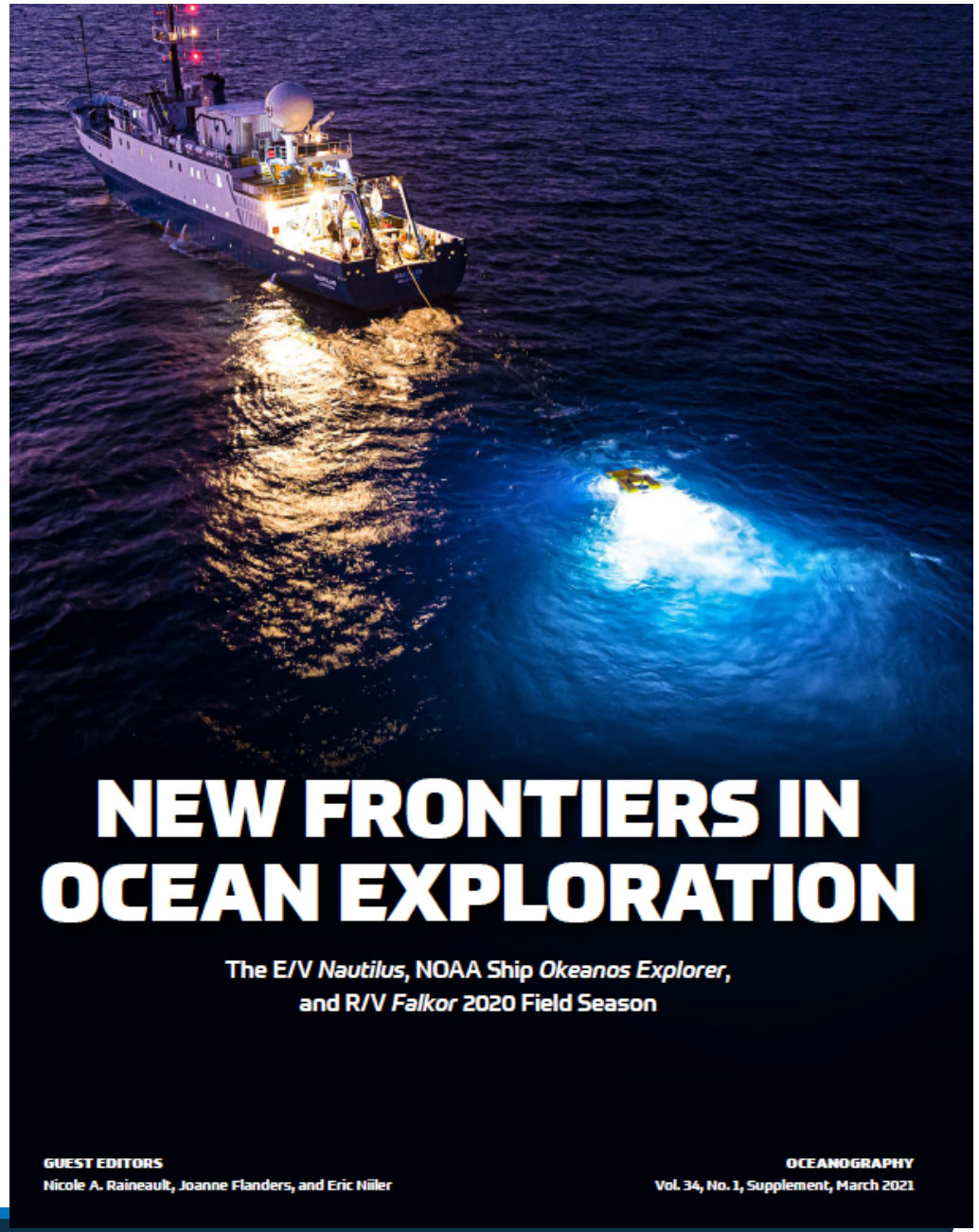

Sarah Duceschi


Jacob Lilly


Larry Mullins


Victoria Simmons


Shannon Agler



BATTELLE

It can be done

800.201.2011 | solutions@battelle.org | www.battelle.org