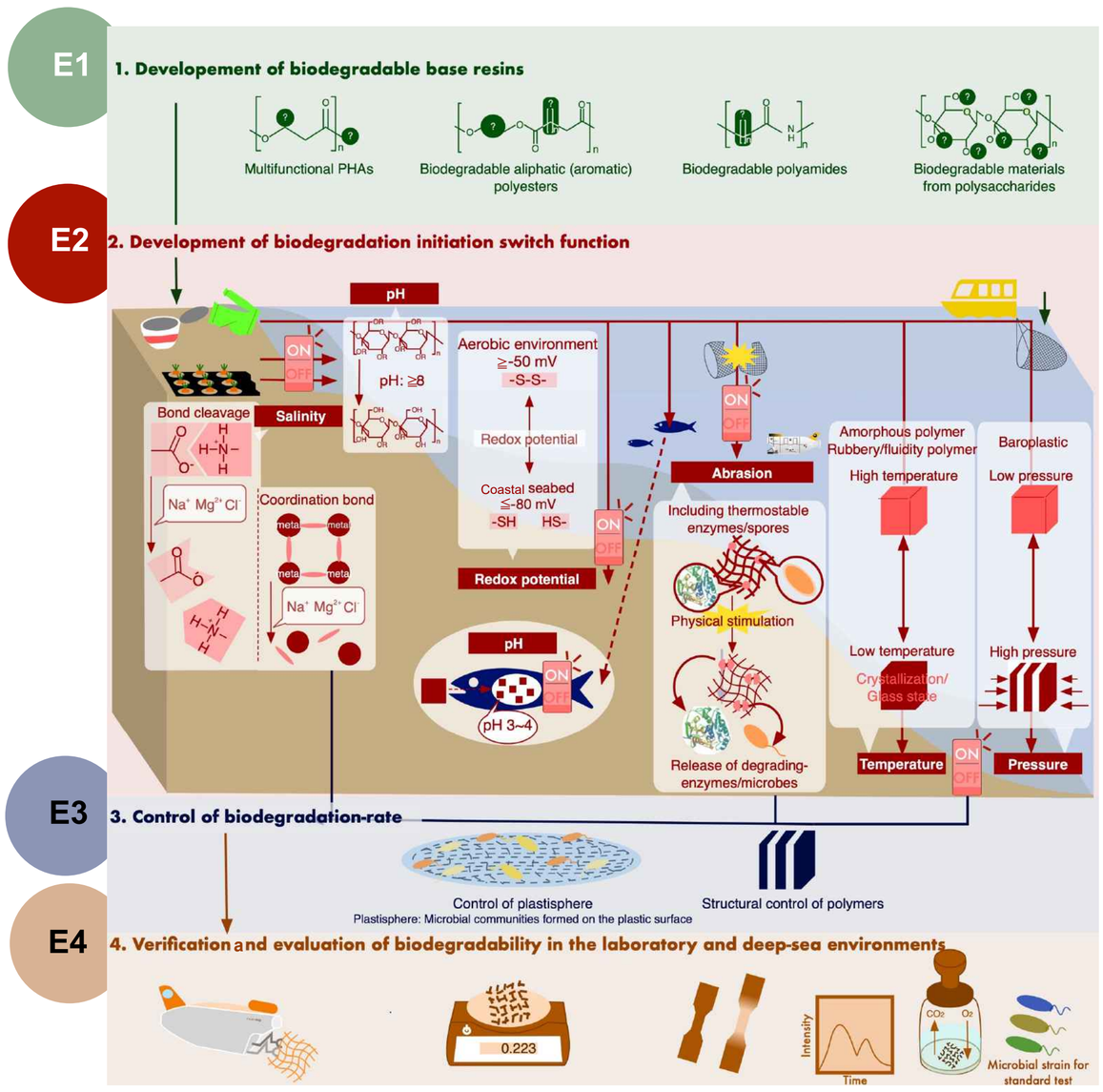
No： A-12-1E
PJ： R & D of marine biodegradable plastics with degradation initiation switch function
Theme：Project overview
Organization： Gunma Univ, U Tokyo, Tokyo Tech, RIKEN, JAMSTEC
Contact： Gunma Univ Ken-ichi Kasuya(kkasuya@gunma-u.ac.jp)

E1 E2 E3 E4

Overview and goal of our project

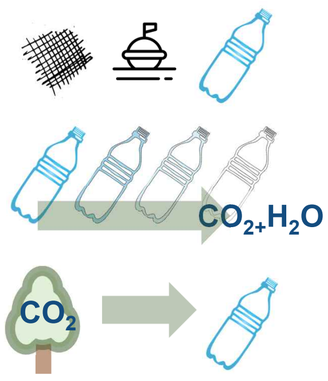


This project has the following goals for social implementation of development technology.

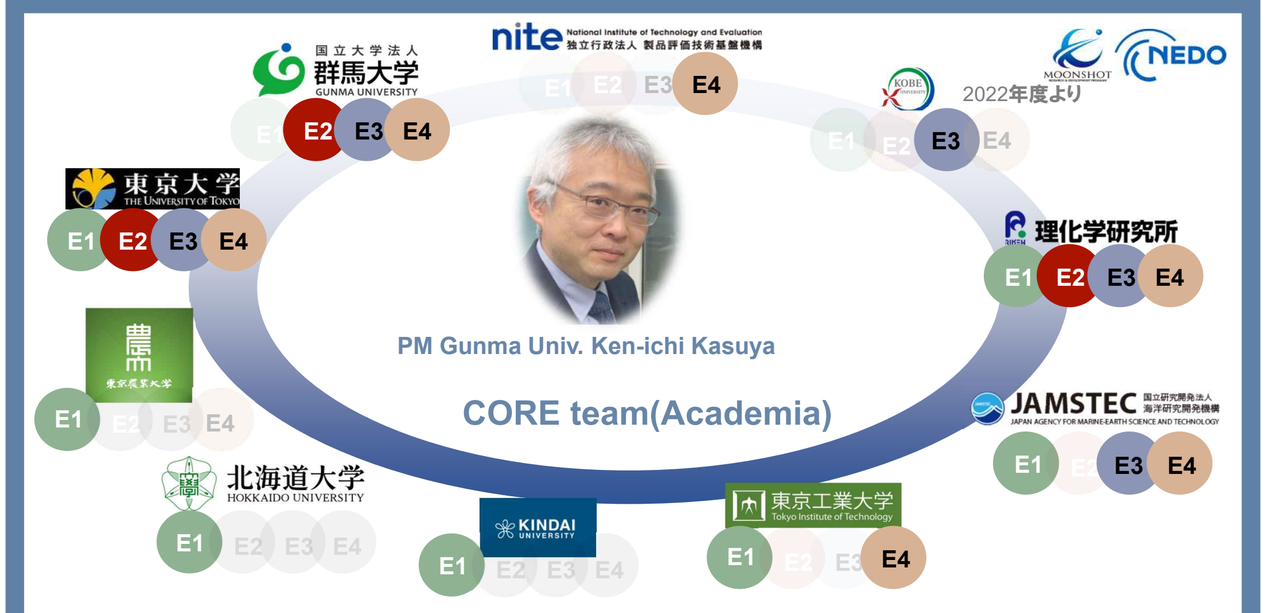
① We create three or more new marine biodegradable plastics that exhibit 90% biodegradability in seawater at 30 ˚C in six months after the switching function exerts.

② We demonstrate the biodegradability of these new marine biodegradable plastics having the switching function in marine environments, including deep sea.

③ We create new marine biodegradable base materials made from biomass and carbon dioxide.



Organization of R & D



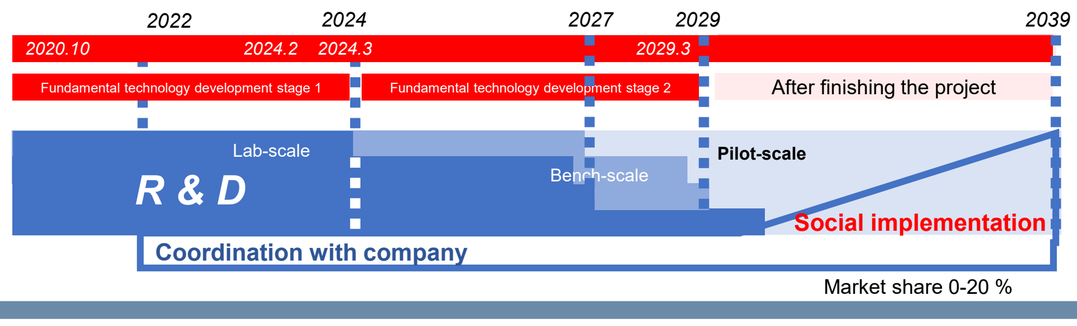
SI 日清紡ケミカル株式会社　SI kaneka

SATELLITE teams (Companies and Academia)

Since 2022, academic-industrial satellite teams are formed to accelerate the social implementation of outcomes developed by the core team.

PM promotes the systemization of elemental technology, reorganizes the teams, and selects the themes to maximize the accomplishments.

Time schedule for R & D



International cooperation

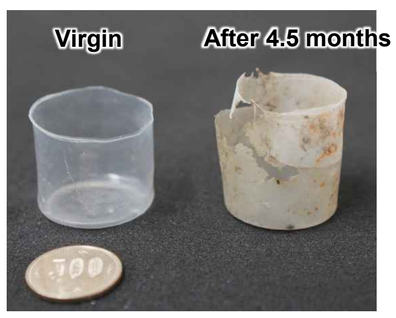
We evaluated degradability of biodegradable plastics in the ocean surface layer in cooperation with the National Oceanic and Atmospheric Administration (NOAA).
Buoys installed in the Great Pacific garbage patch is used as a test site for degradation of biodegradable plastics. When the buoys are replaced every year, biodegradable plastics are collected and newly installed. The degradability of biodegradable plastics in the marine surface environment is verified as part of international joint research.

Science and technology dialogue with the public

As part of GIGA School x Deep Sea, a new biodegradable material was installed 855 m off Hatsushima Island with more than 24,000 elementary school students and the Minister of MEXT via a live online broadcast.

Media appearance

- TOYOBO Column: "Biodegradable Plastic R&D Leading to Solutions to Marine Debris Problems: Now and the future”
- Gunma Kankyo Keizai Forum 2022 20220225
- Biodegradable Plastic Standardization Consortium 20220119
- NTV Sukkiri 20220608
- NBC Radio program 20220622
- Gunma Future Innovation Conference 20220623
- Lecture on contemporary issues in Ibaraki prefecture 202107
- Shibukawa City Citizen's Environmental University 20201115



Above：The material before and after 4.5 months in deep-sea.
Right：News site reporting the results of the material developed in this project and demonstrating on-site biodegradability.


深海から新型プラ引き揚げ　海中で水とCO2に自然分解

Marine debris @Yokosuka

Fish net on beach @Sendai

No： A-12-2E
PJ： R & D of marine biodegradable plastics with degradation initiation switch function
Theme：*E1*, R & D of marine biodegradable base resins that can introduce switch function
Organization： U Tokyo, Tokyo Tech, JAMSTEC, RIKEN
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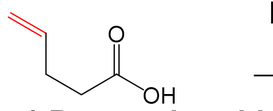
E1 E2 E3 E4

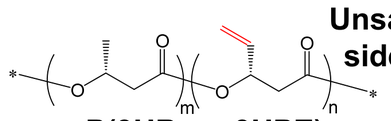
Development of multifunctional microbial polyester：Base materials for installing switching functions

Glucose
Carbon source +



4-Pentenoic acid

Recombinant *E. coli*



P(3HB-*co*-3HPE)

Unsaturated side chain

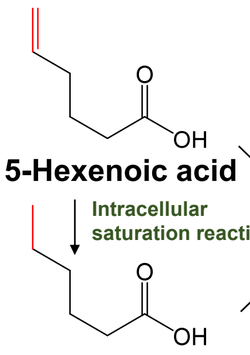
Installing switch function

Flask culture(2L)

Extraction

Synthesize 200 g of 3H4PE polymer
→To install switch function

Sugars +



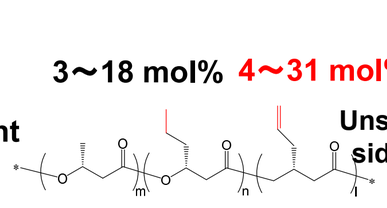
5-Hexenoic acid

Intracellular saturation reaction

Hexanoic acid

Recombinant *R. eutropha*

3～18 mol% 4～31 mol%



P(3HB-*co*-3HHx-*co*-3H5HE)

Unsaturated side chain

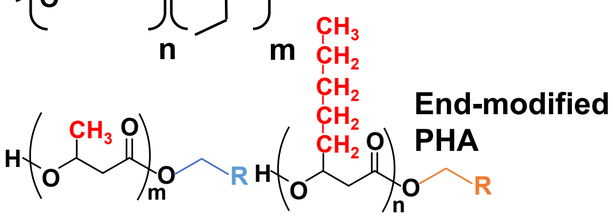
Installing switch function

(A) P(3HB-*co*-4.9 mol% 3H5He-*co*-5.2% 3HHx)

(B) P(3HB-*co*-13.9 mol% 3H5He-*co*-16.3% 3HHx)

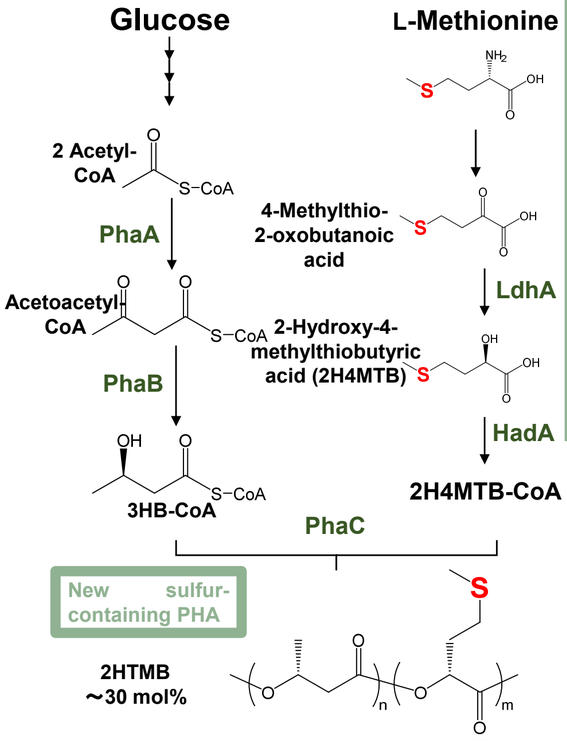
Other materials

P(3HP)-b-P(2HB)
High toughness material

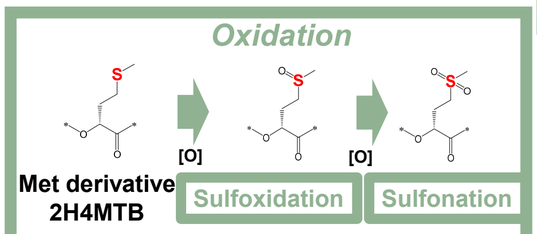


End-modified PHA

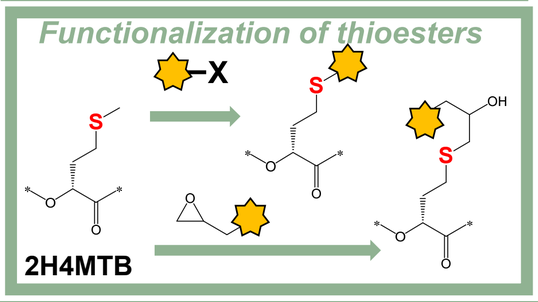
Glucose
L-Methionine



Oxidation



Functionalization of thioesters

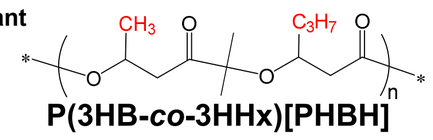


Development of efficient synthesis method for new PHA from CO_2

CO_2

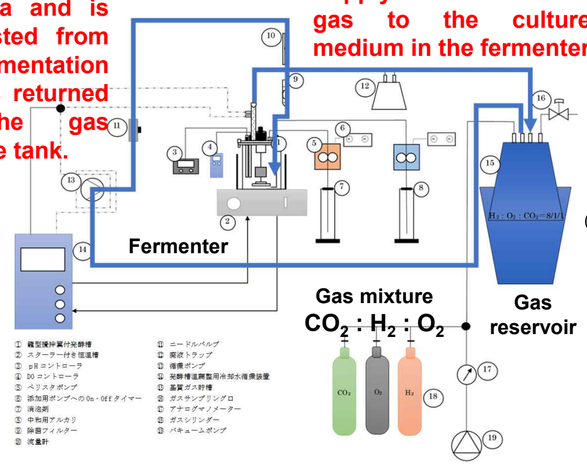
The gas that is not used by the bacteria and is exhausted from the fermentation tank is returned to the gas storage tank.

Recombinant strain



P(3HB-*co*-3HHx)[PHBH]

Supply of raw material gas to the culture medium in the fermenter



Closed circulation gas culture system

① PHBH productivity of various recombinant strains (flask culture)

Strain / Plasmid	Dry cell wt. (g/l)	PHBH Content (wt%)	Composition (mol%) 3HB	3HHx
C.necator H16 (wild-type)	17.16	68.2	100.0	0
MF01/ pBPP-ccr$_{Me}$J4a-emd	12.18±0.40	64.0±3.4	94.8±1.1	5.3±1.1
MF01ΔB1/ pBPP-ccr$_{Me}$J4a-emd	10.65±1.35	61.7±4.6	52.3±6.2	47.7±6.2
MF01/ pBPP-ccr$_{Me}$JAc-emd*	11.22±2.67	64.6±8.1	88.7±6.4	11.3±6.4
MF01ΔB1/ pBPP-ccr$_{Me}$JAc-emd	8.52±1.00	67.8±1.8	87.1±2.3	11.1±1.3

* 3HHx 10mol% PHBH is known to have the best physical properties(~2020)

② PHBH production test by jar culture of *C. necator* strain MF01/JAc

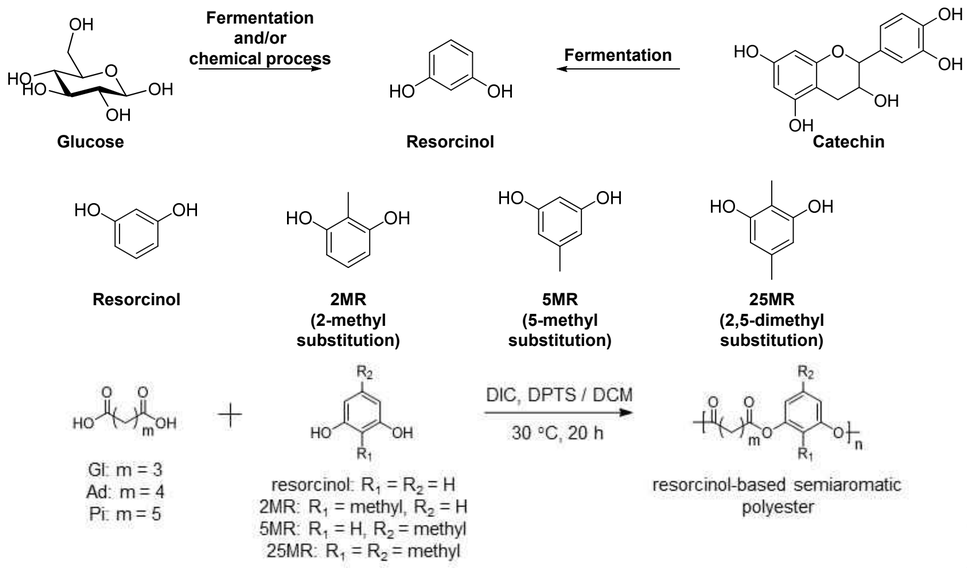
Dry cell (g/L)	PHBH (g/L)	3HB (mol%)	3HHx (mol%)	Culture time (h)	Productivity (g/L/h)
61.4	51.5	94.6	5.4	205	0.300

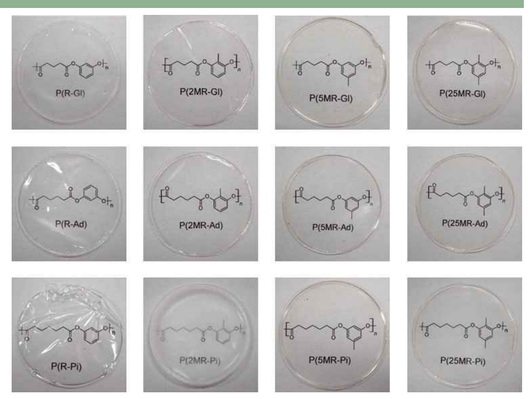
- Search for mineral salt medium composition suitable for PHBH biosynthesis and control of inorganic nutrient concentration during culture
- Productivity improvement by improving fermentation tank agitation performance, etc.

71.0	58.4	86.2	13.8	119	0.594

(Highest value as of November 2022)

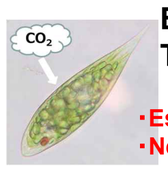
Synthesis of polyesters from aromatic diols with resorcinol back-bone structure





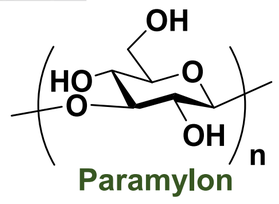
Succeeded in synthesis of polyesters from aromatic diols with resorcinol back-bone structure by using carbodiimide compounds.
The products with high molecular weight (Mn>24kg/mol) could be obtained.

Marine biodegradable plastics produced from polysaccharides



Extraction
Thermo-processing
・Esterification
・New processing procedure

Paramylon

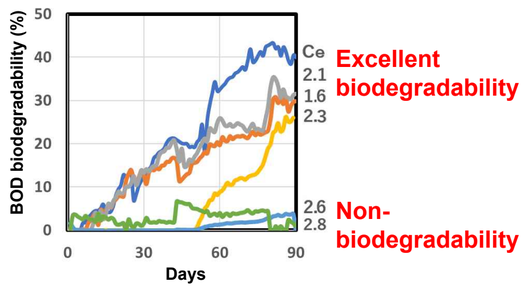


Injection molding
- Resistant to acids and alkalis
- Better impact strength > PP

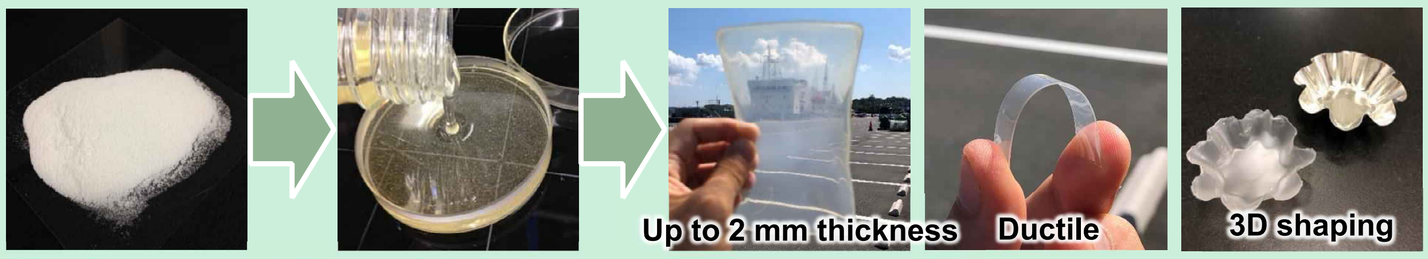
Melt-spun fibers
- Processable without additives
- High-strength

BOD biodegradation test

- Using Seawater from Tokyo Bay
- Successful development of new high-performance materials with controlled marine degradability from polysaccharides



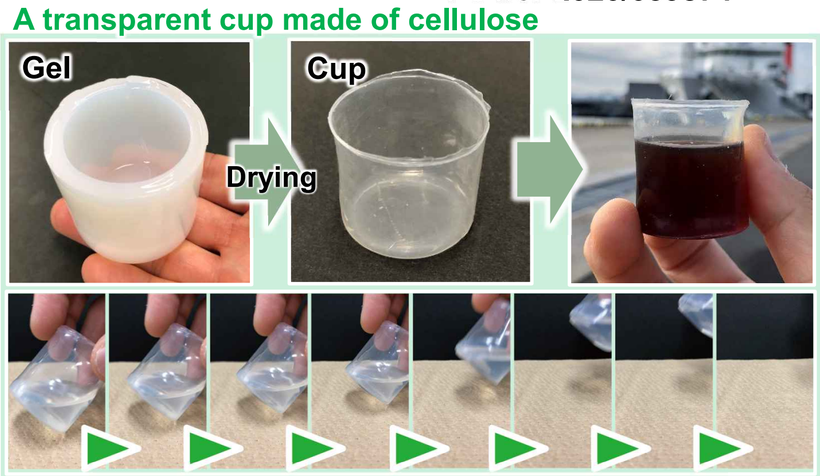
Development of new cellulose-based transparent materials



Dissolving, coagulating, and drying cellulose gives transparent paperboard.
PCT/JP2020/039874

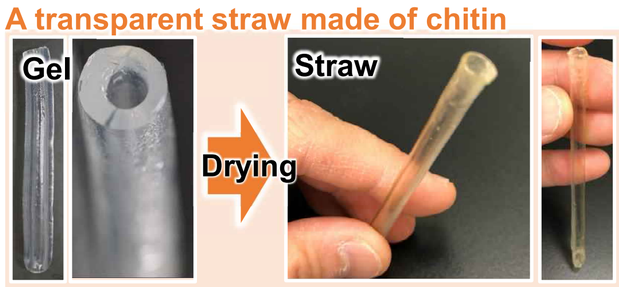
Compositionally identical with paper but more functional.

A transparent cup made of cellulose



The cup holds water without inner film, which is necessary for the conventional paper cup.

A transparent straw made of chitin

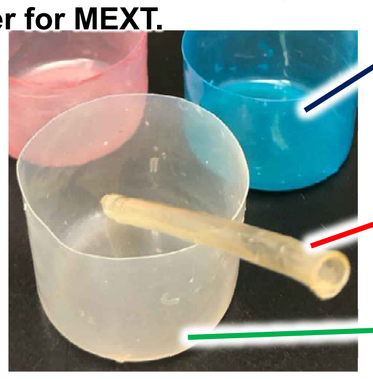


The fragileness of chitin was overcome by the improved molding process.

Proof of concept of the transparent cup made of cellulose

Deployment of transparent cup made of cellulose at the deep-sea floor was broadcasted to the elementary schools in Japan in the presence of minister for MEXT.

Appeared in news23 (TBS) and 16 news papers.



By the improved shaping process, the preparation of materials entirely made of pristine cellulose or chitin was successful.

No： A-12-3E
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Theme：*E2*, Development of switching function to start biodegradation
Organization： U Tokyo, RIKEN, Gunma Univ
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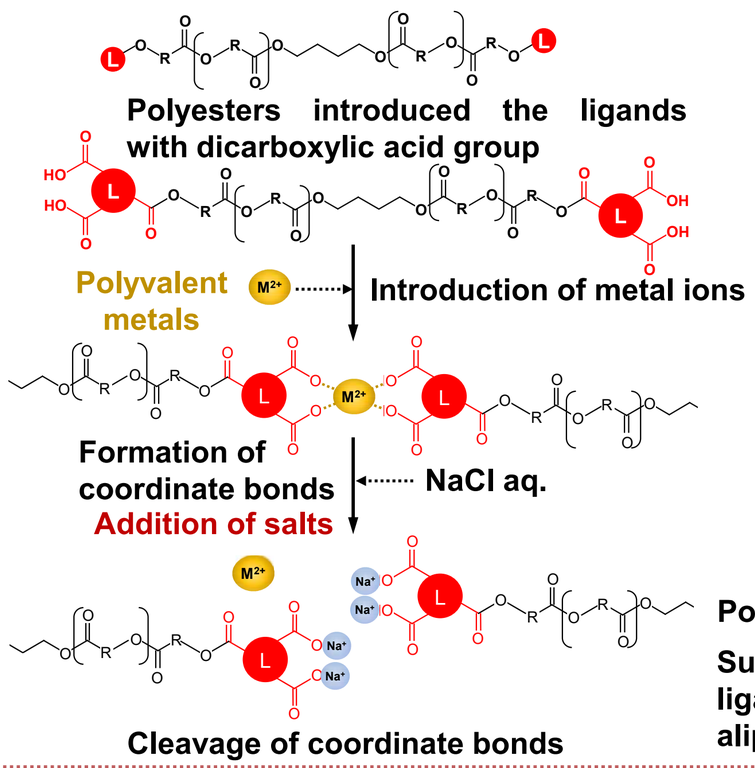
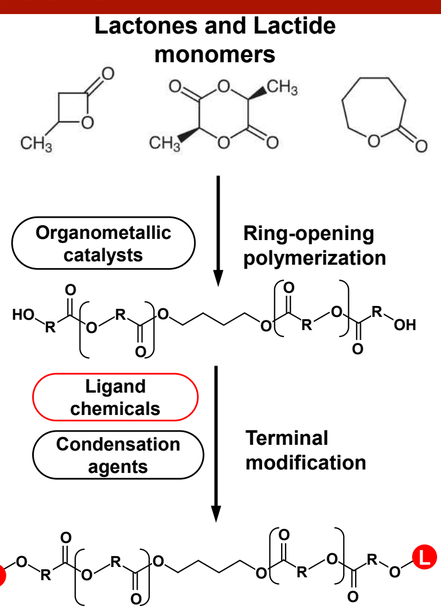
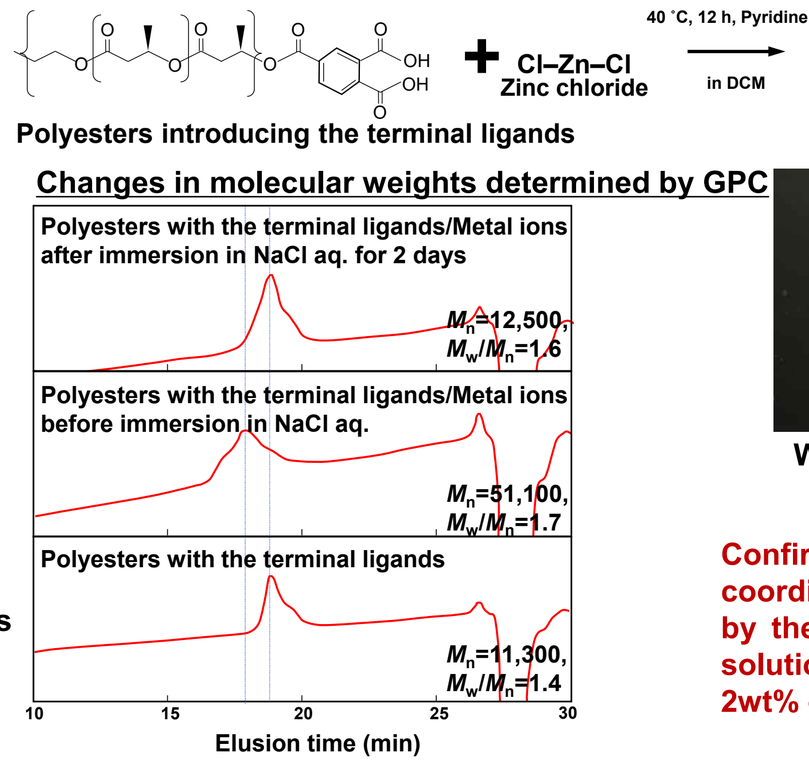
E1 E2 E3 E4

Switching triggered by difference in salt conc.



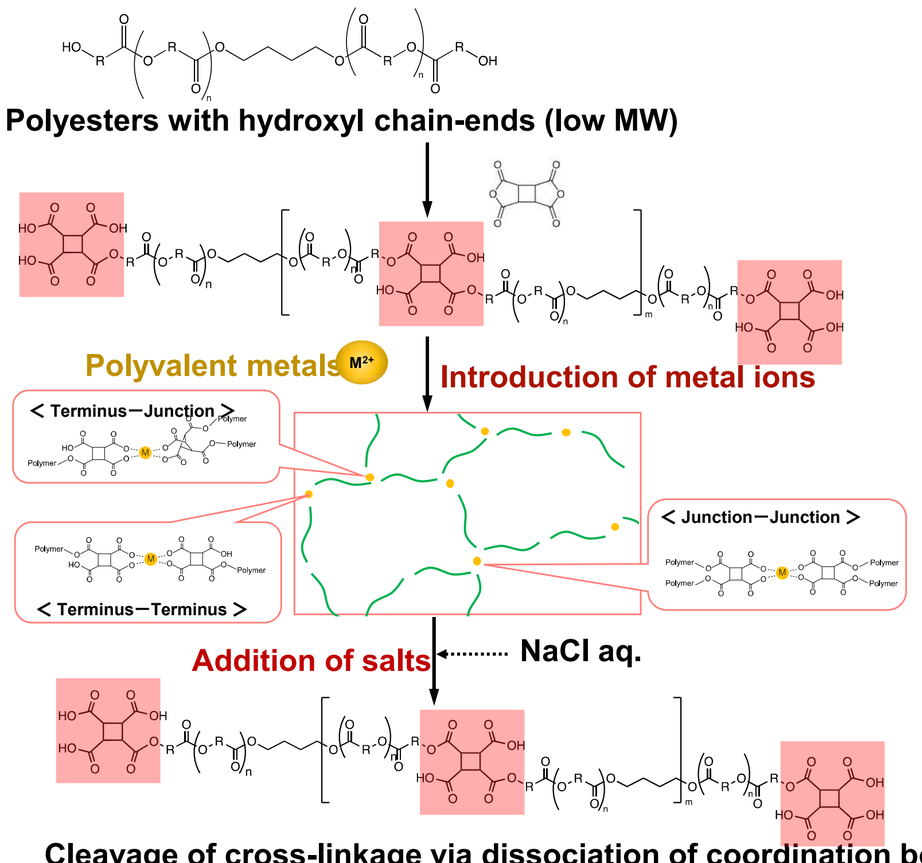


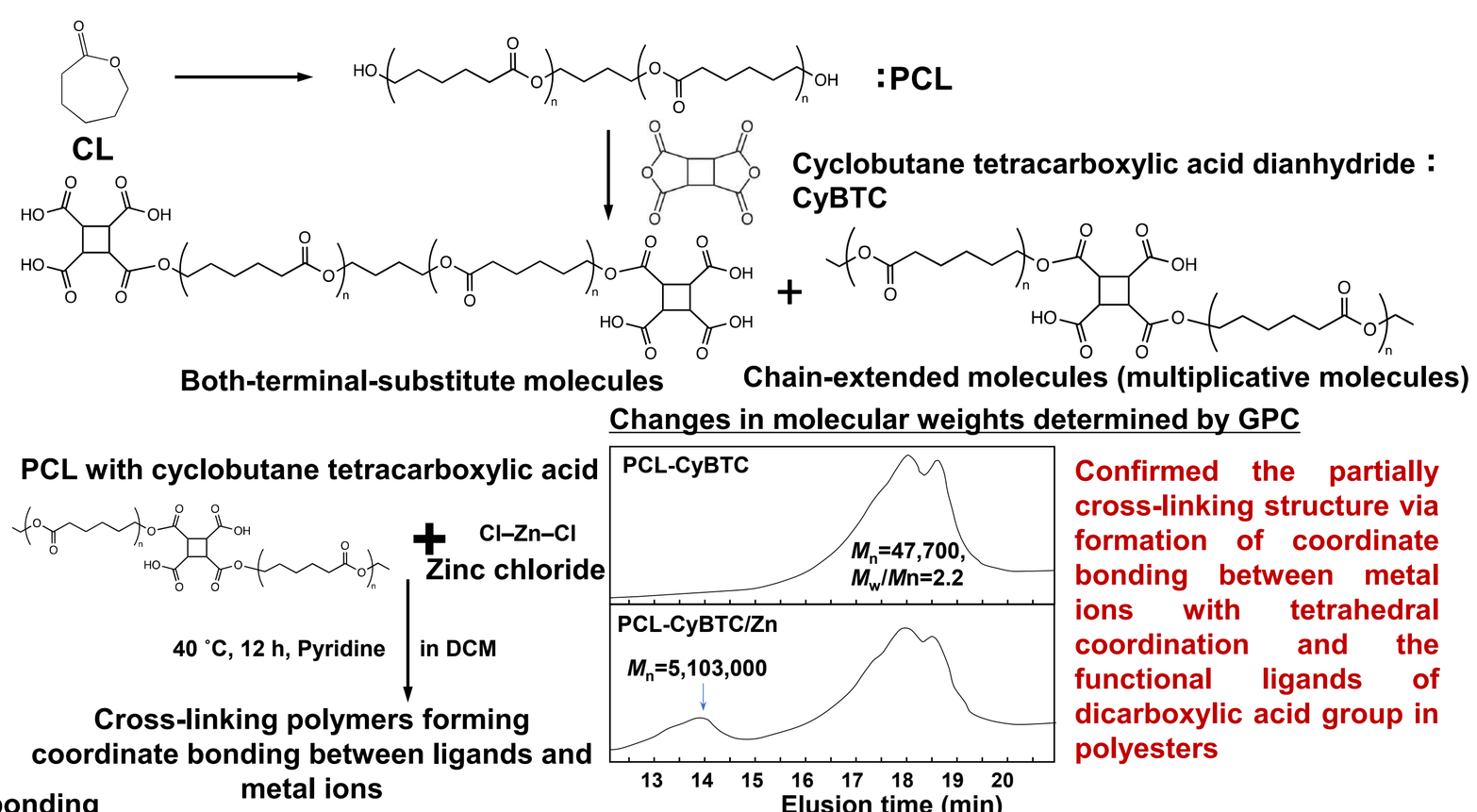
Succeeded in introduction of ligands into the chain-ends of aliphatic polyesters



Linear polymers forming coordinate bonding between terminal ligands and metal ions

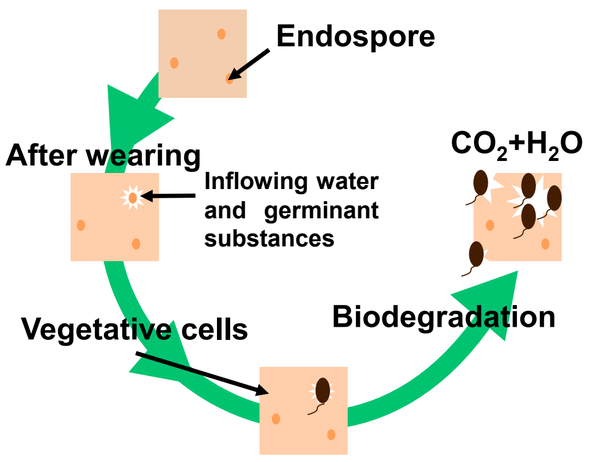
Confirmed the cleavage of coordinate bonding of polymers by the immersion into NaCl aq. solution with a concentration of 2wt% or more.



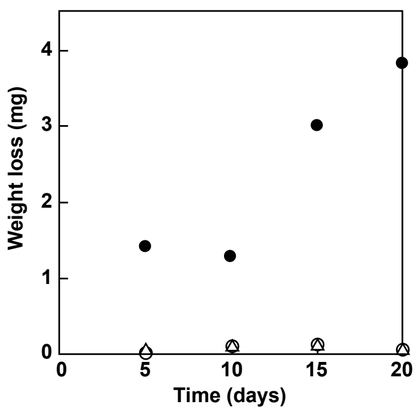


Confirmed the partially cross-linking structure via formation of coordinate bonding between metal ions with tetrahedral coordination and the functional ligands of dicarboxylic acid group in polyesters

Switching triggered by wear (Endospore)

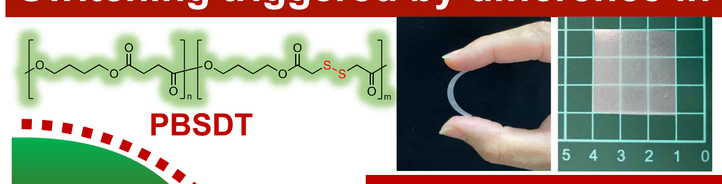


- Degradation is triggered by wear, and biodegradation proceeds as endospores transform to vegetative cells.

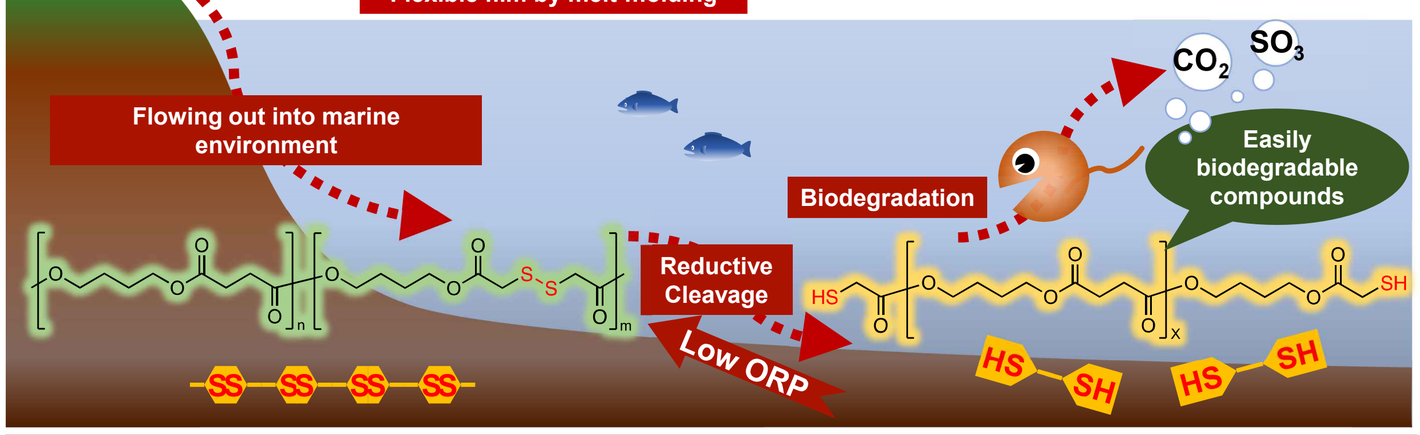


Degradation test of spore-containing PESu ●：Weight loss of spore-containing PESu film with Yeast extract (YE). ○：Weight loss of PESu film with YE. △：Weight loss of spore-containing PESu film without YE.

Switching triggered by difference in ORP

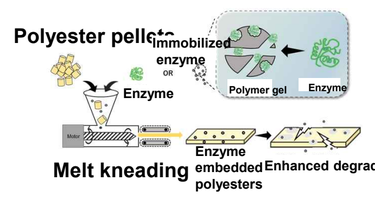


- PBSDT as an analogue of PBSA
- Reductive cleavage of disulfide bonding in low ORP condition
- Biodegradation of the reduced compound in marine

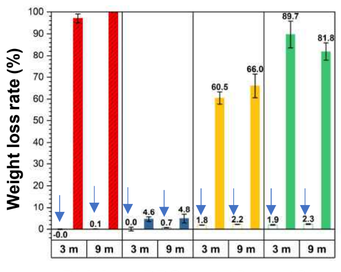


Switching triggered by wear (Enzyme)

Biodegradation triggered by wear and enhanced by embedded enzyme



Enzymes (Lipase, Cutinase, proteinase K) were embedded in biodegradable plastics that show slow degradation in the ocean. Enzymes were immobilized in gel-beads to improve stability against heat molding.

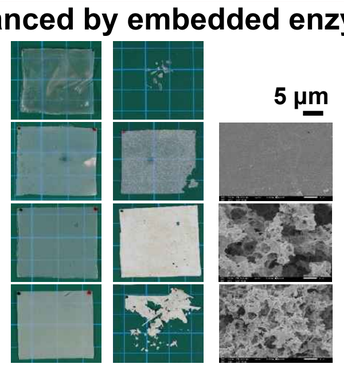


Biodegradation in deep sea (Hatsushima, 15-20 ℃, 3 and 9 month

Without enzyme: no degradation

Enzyme-embedded polyesters: High weight loss (%) and degradation

Enzyme-embedded PLLA
Enzyme-embedded PBS
Enzyme-embedded PBSA
Enzyme-embedded PCL

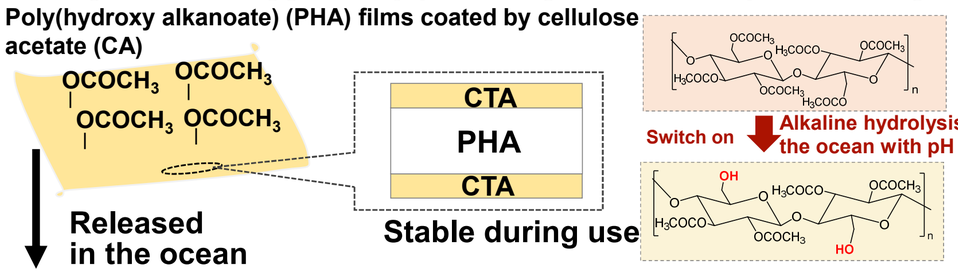


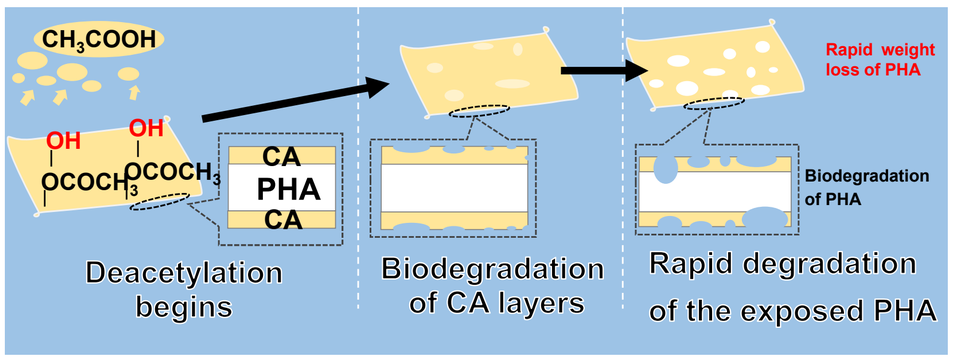
- Enzyme-embedded PLLA, PBS, PBSA, PCL were prepared
- Enzyme-embedded polyesters were successfully degraded in the ocean (Hatsushima, JAPAN)

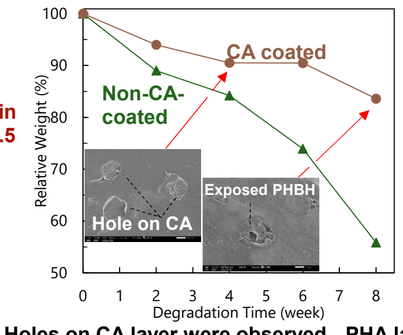
Switching triggered by difference in pH

Biodegradation triggered by pH change and subsequent biodegradation of polysaccharide ester

Poly(hydroxy alkanoate) (PHA) films coated by cellulose acetate (CA)







Holes on CA layer were observed. PHA layer started rapid degradation after CA degradation

- PHA films were coted with cellulose acetate (CA) layer.
- Biodegradation of CA layers was triggered by alkaline hydrolysis in the sea water and subsequent decrease in degree of substitution.
- Degradation did not occur in water with pH 7.

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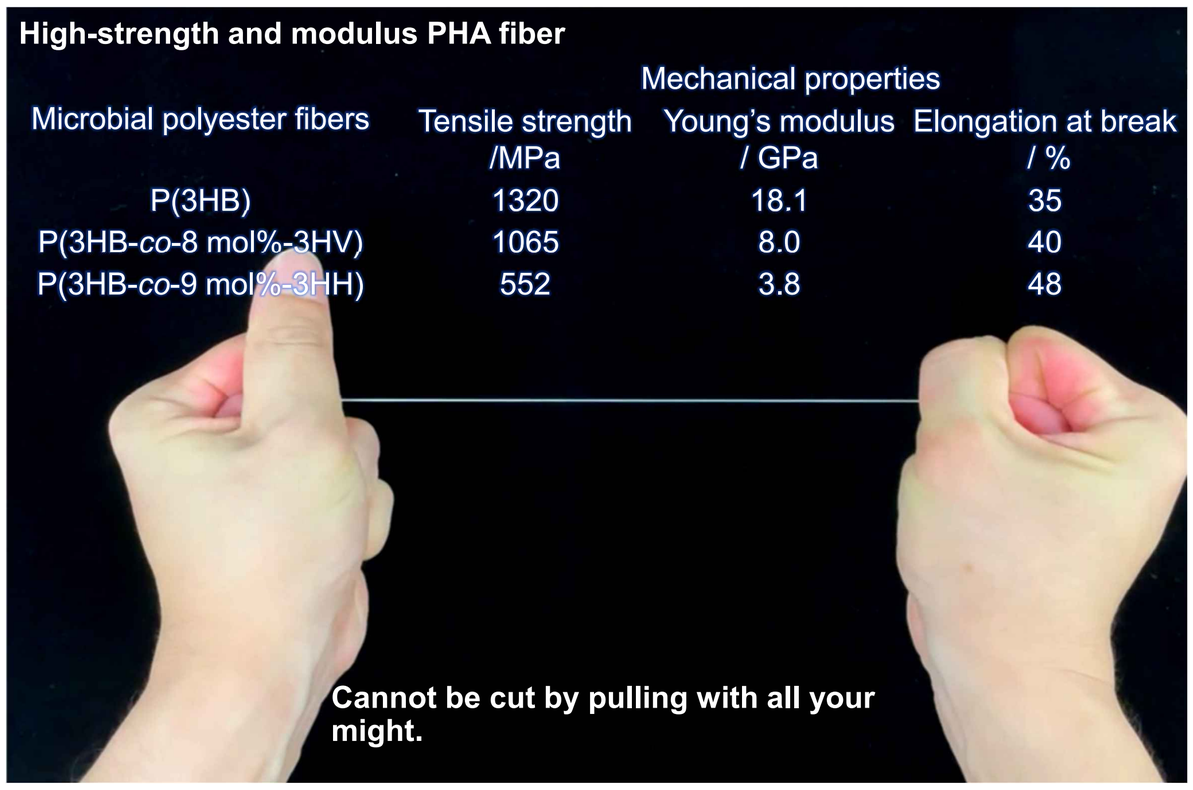
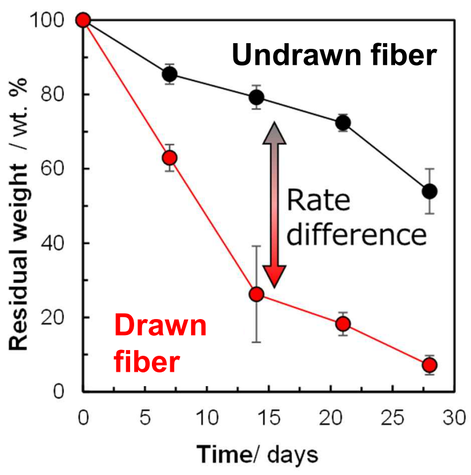
E1 E2 **E3** E4

Biodegradation rate factors from materials science

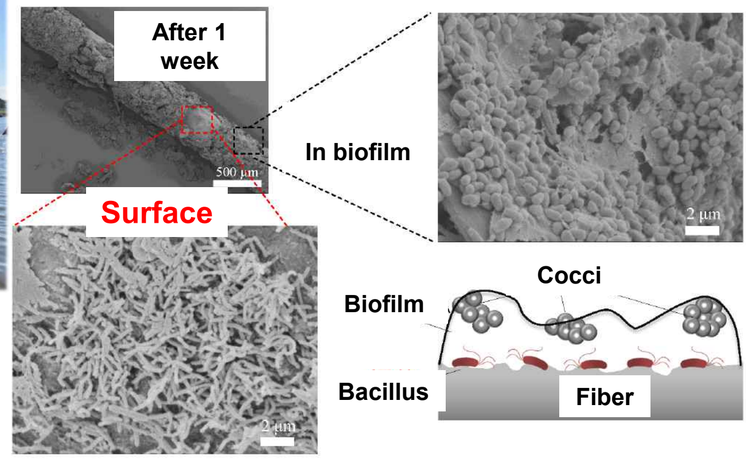
High-strength and modulus PHA fiber

Microbial polyester fibers	Mechanical properties: Tensile strength /MPa	Young's modulus / GPa	Elongation at break / %
P(3HB)	1320	18.1	35
P(3HB-*co*-8 mol%-3HV)	1065	8.0	40
P(3HB-*co*-9 mol%-3HH)	552	3.8	48





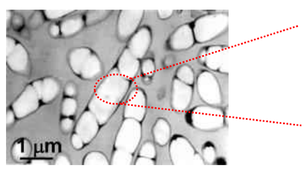
Biodegradation of fiber

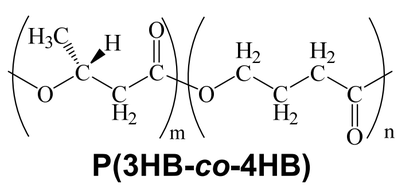


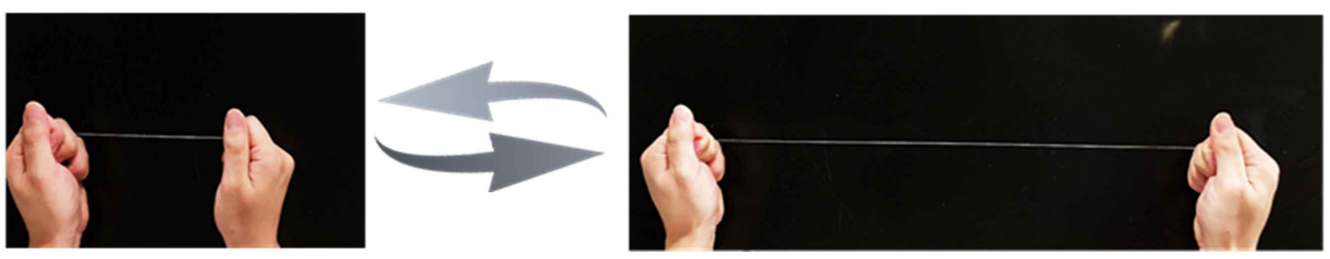
Bacillus on the fiber surface may degrade fiber using the enzyme.

- Degradation rate can be controlled by drawing ratio.
- Degradation rate is related to crystalline morphology.

- Polyesters produced by microorganisms.
- P(3HB-*co*-4HB) in the one of the copolymers.

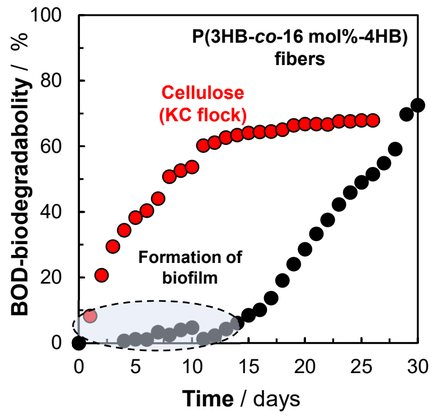


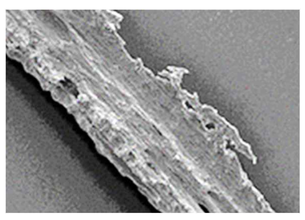




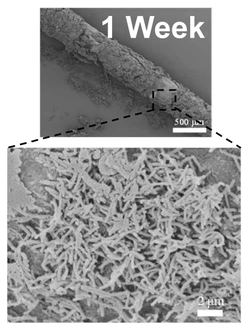
Highly stretchable biodegradable fibers

BOD biodegradability in seawater



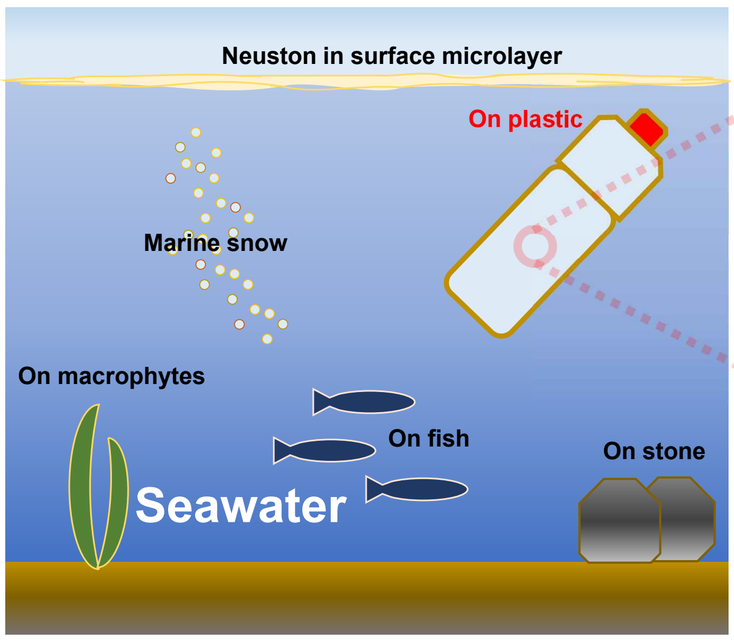


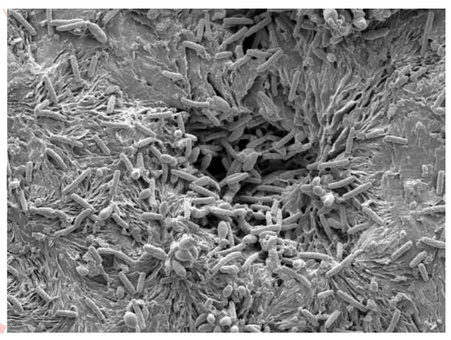
SEM image of the fiber during degradation



Microorganisms on fiber surface

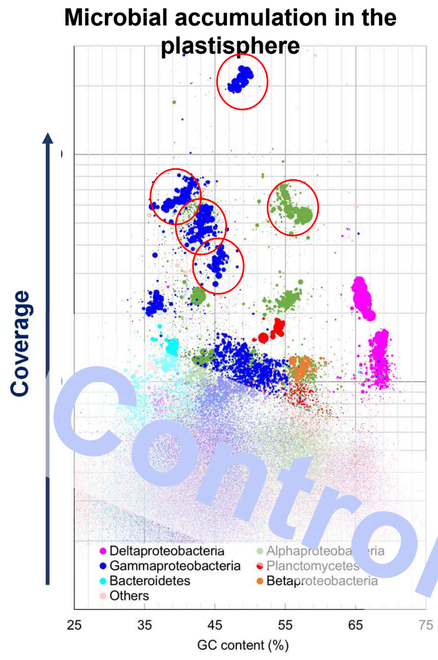
Plastisphere：Microbial flora formed on plastic surface





SEM image of plastisphere formed on the biodegradable plastic surface.

Metagenome analysis



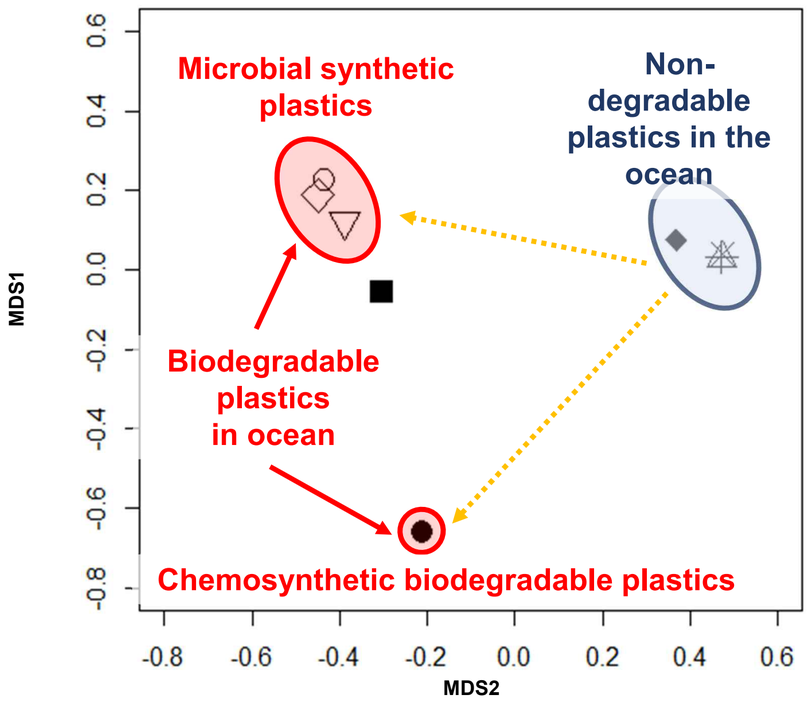
Microbes with high abundance = Genome information of microbes involved in the biodegradation.

Elucidate the biodegradation mechanism of plastic and control the degradation rate.

Also investigate the plastisphere in non-oceanic environments.

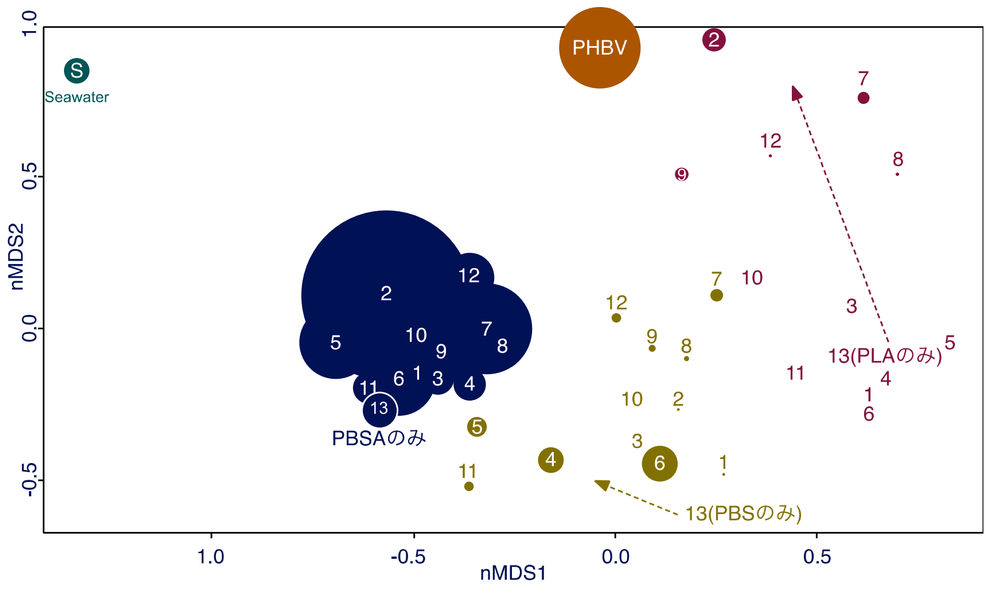
Biodegradable plastics is exposed to pond.

Biodegradation rate control by controlling plastisphere



The plastisphere of Non-marine biodegradable plastics close to that of marine biodegradable plastics.→ Improving biodegradability

Addition of 10% plastisphere control substance candidate to the biodegradable base polymer.
The films were exposed to seawater and investigated weight loss and change in plastisphere.



Non-metric multidimensional scaling (nMDS) based on the Bray-Curtis index. Numbers in the plot indicate the type of substance. The area of the plot shows the biodegradation rate except for seawater.

Plastisphere control substance candidates

	Substance
1	CE
2	A
3	CH
4	P
5	SH
6	HH
7	C
8	C
9	AN
10	AC
11	P
12	Y
13	Negative control

Effect on increase in the degradation rate.

PBSA
No.2、No.5、No.6、No.7

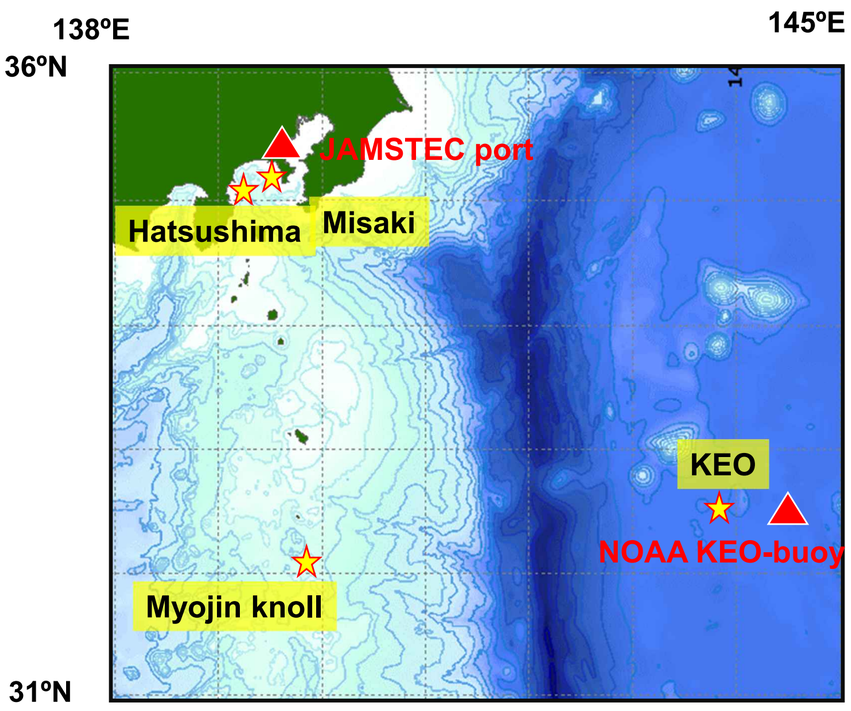
PBS
No.5、No.6

PLA
No.2、No.7、No.9

No： A-12-5E
PJ： R & D of marine biodegradable plastics with degradation initiation switch function
Theme：*E4*, Validation and evaluation of biodegradability in laboratory and deep-sea environments
Organization： JAMSTEC, U Tokyo, Gunma Univ
Contact: Gunma Univ Ken-ichi Kasuya (kkasuya@gunma-u.ac.jp)

E1 E2 E3 **E4**

In situ biodegradation tests of novel materials



- We carried out 6 cruises to test the biodegradability of newly developed materials on the deep-sea floor from 2020 to 2022. This is the only project that is testing biodegradability on the deep-sea floor in situ, where large amount of plastic debris are accumulating.
- We have also started biodegradability test at the surface of the North Pacific pelagic site.
- The recovered materials were examined with different chemical and physical tests, together with meta-omics approaches of the attached biofilms.


Deployment and recovery with the manned submersible


Plastic chambers deployed on the deep-sea floor

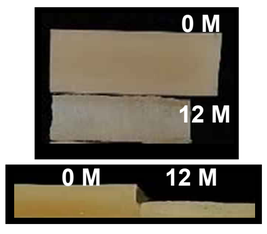

Plastic chambers deployed on the mooring buoy

In situ biodegradation tests: in shallow water

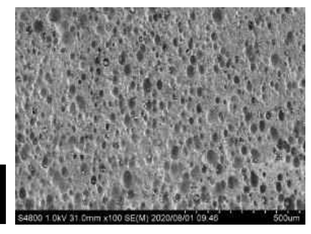
Biodegradation of biodegradable plastics in shallow water

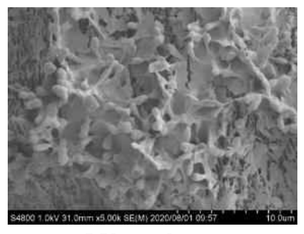
	Length (mm)	Wide(mm)	Thickness (mm)	Weight (g)
0 Month	30.0	10.0	4.0	1.30
12 Month	25.5	7.5	2.2	0.39
Reduction rate	15%	24%	45%	70%


Setting

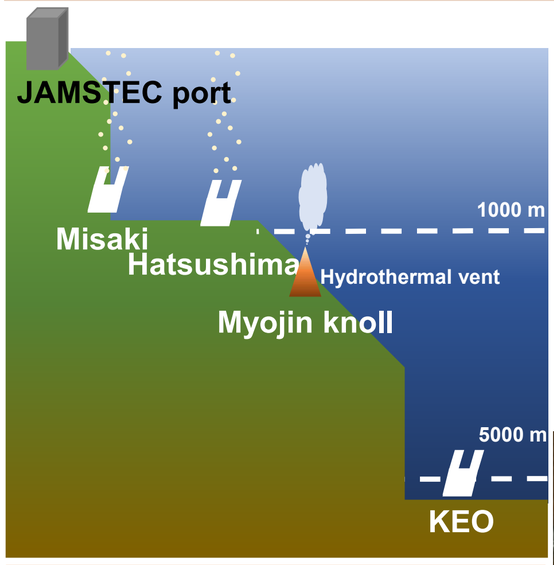


Size

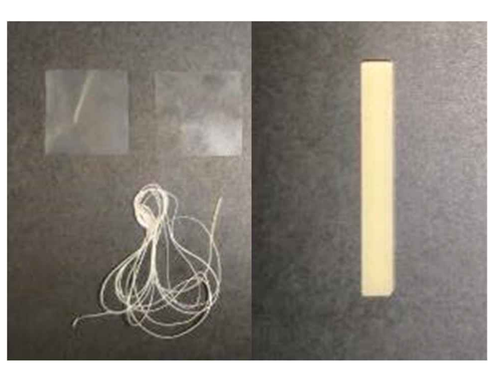

Surface


Microbes

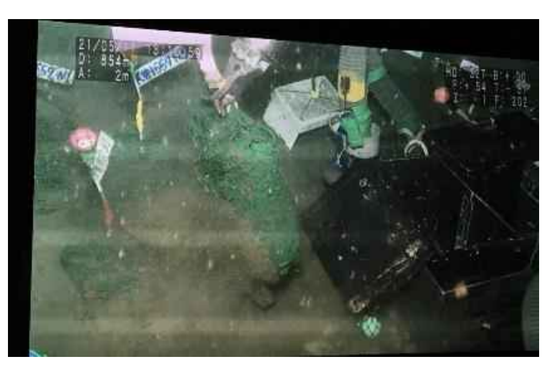
In situ biodegradation tests in deep-sea



	Salinity	Temp.	DO
JAMSTEC port	28-29	23-26	
Hatsushima	34.5	3.6	1.24
Misaki	34.3	4.4	1.46
Myojin knoll	34.3	4.6	1.50
KEO	34.7	1.6	3.6-3.7


Biodegradable plastics


Samples after 4 months of installation


Recovery using a robotic arm


Chamber filled with biodegradable plastics


Recovery of samples and deep-sea water


Collection of seabed soil

In vitro biodegradation tests of novel materials

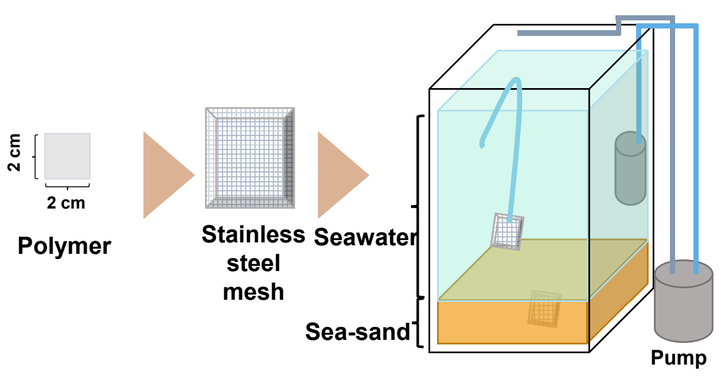

BOD biodegradation testing

Biodegradable plastics + O_2 ▶ CO_2
By microbes

$$Biodegradability\% = \frac{O_2}{Th\,OD} \times \frac{100}{}$$

O_2: Biological oxygen demand (BOD) used for catabolism of compounds

ThOD： Theoretical oxygen demand



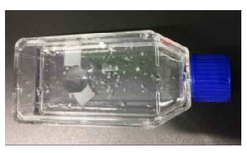
Tank experiment

- Weight loss
- Morphology of surface
- Mechanical properties
- Plastisphere analysis

Optimization of *in vitro* test condition

① Biofilm development for obtaining plastic degraders

Laboratory (4 months)
PBSA and PBAT films were put in sea water of 4 sites.



In the sea (5 months)
(Hiroshima, 10 m-depth)
Films of PBSA, PBS, PBAT, and PCL with film mounts were fixed in the fixture.

- PBSA/PBAT degraders (230 strains) were isolated from plastisphere.

② Degradation property analyses of degraders on each material and degrader selection for rapid test method


Agar plate with emulsified plastics.

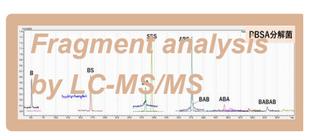
Isolation


Plastic film and bacteria in artificial seawater.

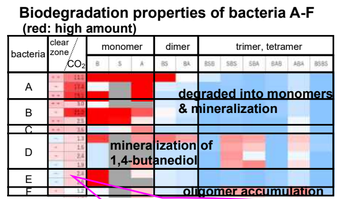
Culture

Gas
Liquid

CO_2 detection by GC



Biodegradation properties of bacteria A-F (red: high amount)



clear zone development: -, CO_2 production: +

- Degradation property of each degrader has been analyzed by clear zone development, CO_2 production and accumulated degradation products.