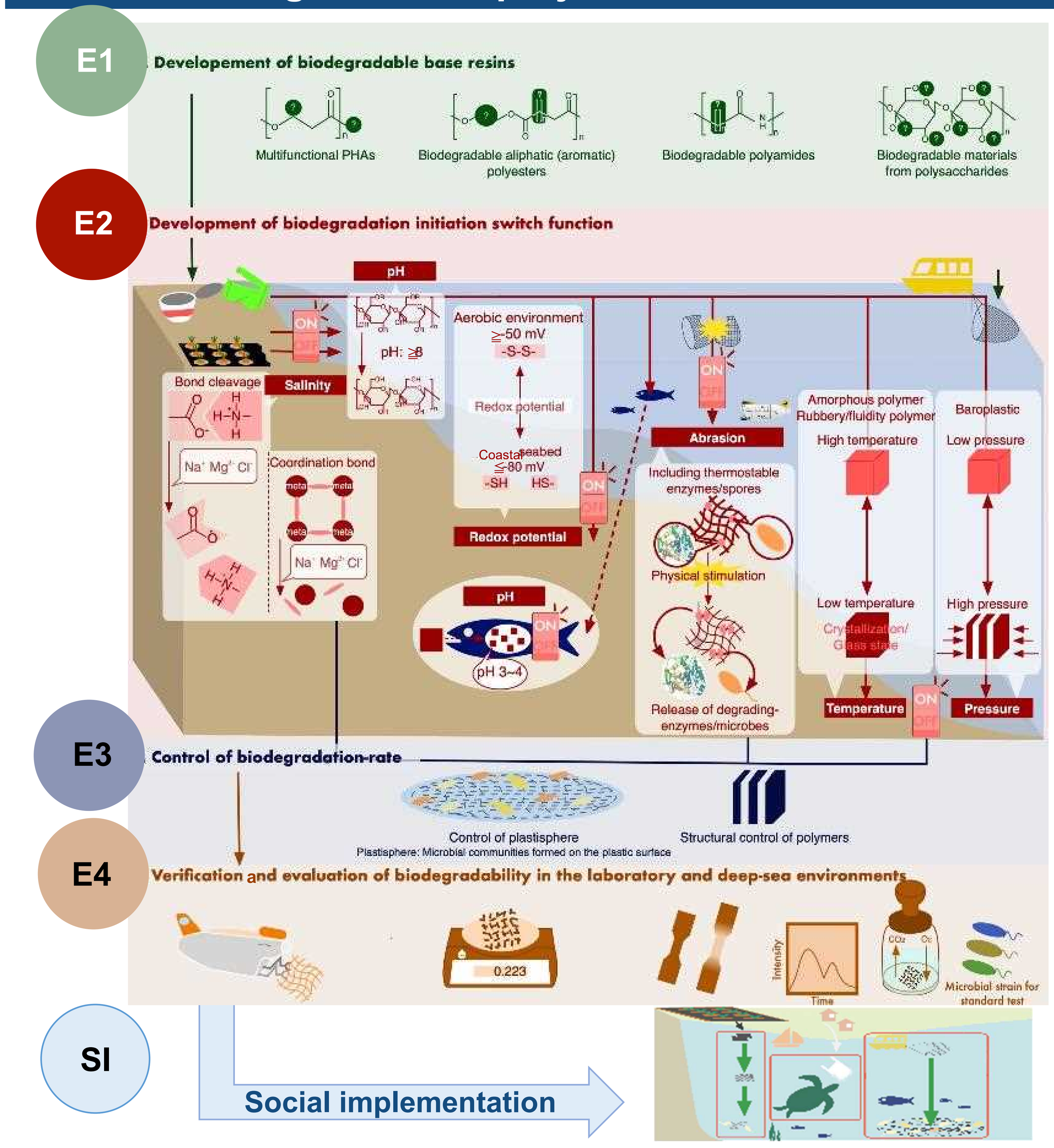
No.: A-16-1E
PJ: R & D of marine biodegradable plastics with degradation initiation switch function
Theme: Project overview
Organization: Gunma Univ, U Tokyo, Tokyo Tech, RIKEN, JAMSTEC
Contact: Gunma Univ Ken-ichi Kasuya(kkasuya@gunma-u.ac.jp))

E1 E2 E3 E4 SI

Overview and goal of our project

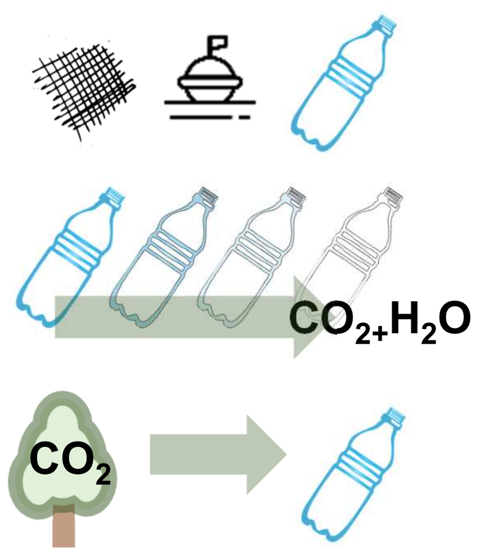


This project has the following goals for social implementation of development technology.

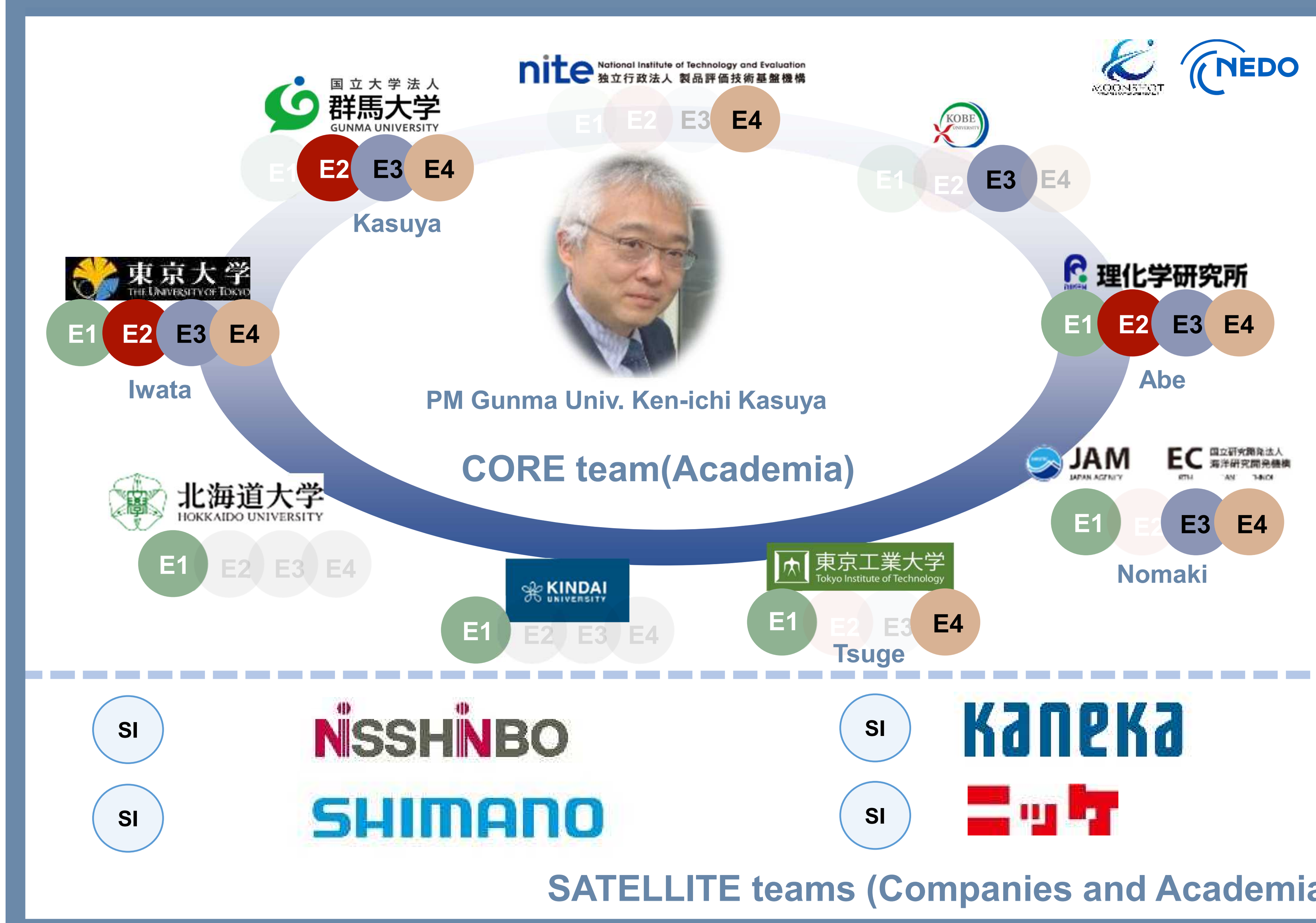
① We create three or more new marine biodegradable plastics that exhibit 90% biodegradability in seawater at 30 ˚C in six months after the switching function exerts.

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

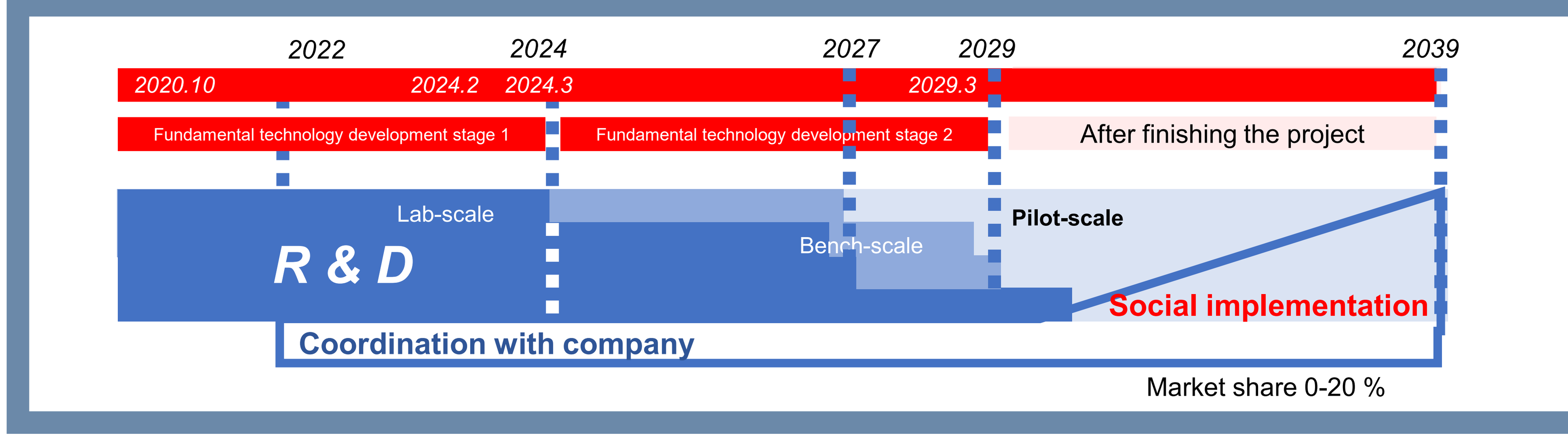


PM promotes the systemization of elemental technology, reorganizes the teams, and selects the themes to maximize the outcomes.

Other external cooperating companies

日本カーリット株式会社 Japan Carlit Co., Ltd.　DAICEL　etc•••

Time schedule for R & D



International cooperation

Biodegradable plastic ocean surface mooring experiment on the NOAA observatory buoy

Patrick Berk, Research Scientist,
National Oceanic and Atmospheric Administration (NOAA)

We evaluated degradability of biodegradable plastics in the ocean surface layer in cooperation with the National Oceanic and Atmospheric Administration (NOAA).

Evaluation of biodegradation of biodegradable plastics on tropical mangrove forests.

Sudesh Kumar, Professor,
Universiti Sains Malaysia (USM)

Assessing the degradability of marine biodegradable plastics in marine plastic debris hotspots.

Evaluation of biodegradation of materials developed in the PJ in mangroves（Right）
Plastic debris in mangroves(Left)

Joint implementation with NEDO Moonshot ITO PJ

- Biodegradation assessments and Publicity Activities in Southeast Asia.
  - Degradation testing of marine biodegradable plastics (Malaysia, Thailand, and Indonesia)
  - Workshops in Thailand and Malaysia (Fall 2024 scheduled)

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.

A special exhibition, "The Sea: The Source of Life," was held at the National Museum of Nature and Science, Tokyo from July 15 to October 9, 2023. The exhibition raised awareness of the marine plastic problem and appealed to the public to reduce environmental impact through biodegradable plastics. During the exhibition, 200,000 people attended.

Major media appearances

- BSFuji Galileox 20231126
- NHK Science ZERO 20231125
- Aichi Television GO JISUTA 20230821
- Fujitsu DESIGN SPECTACLES 20230803,10
- G7 Digital and Tech Minister's in Takasaki Gunma 20230430
- NTV Sukkiri 20220608

No.: A-16-2E

PJ: R & D of marine biodegradable plastics with degradation initiation switch function

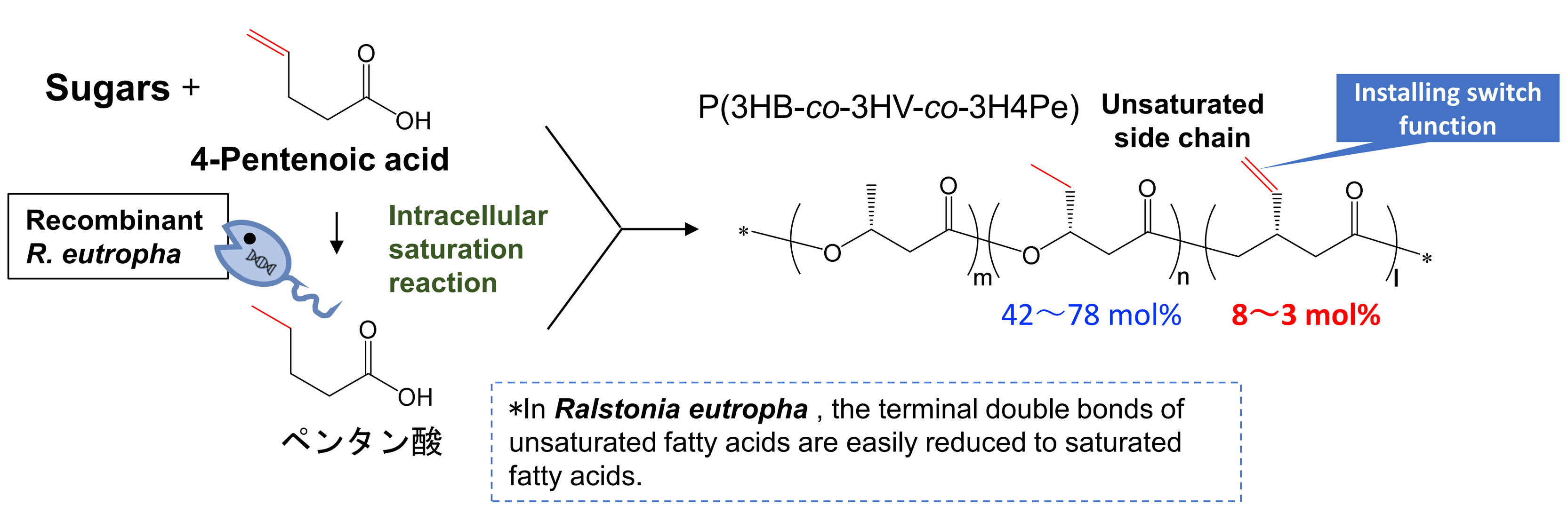
Theme: *E1*, R & D of marine biodegradable base resins that can introduce switch function

Organization: U Tokyo, Tokyo Tech, JAMSTEC, RIKEN

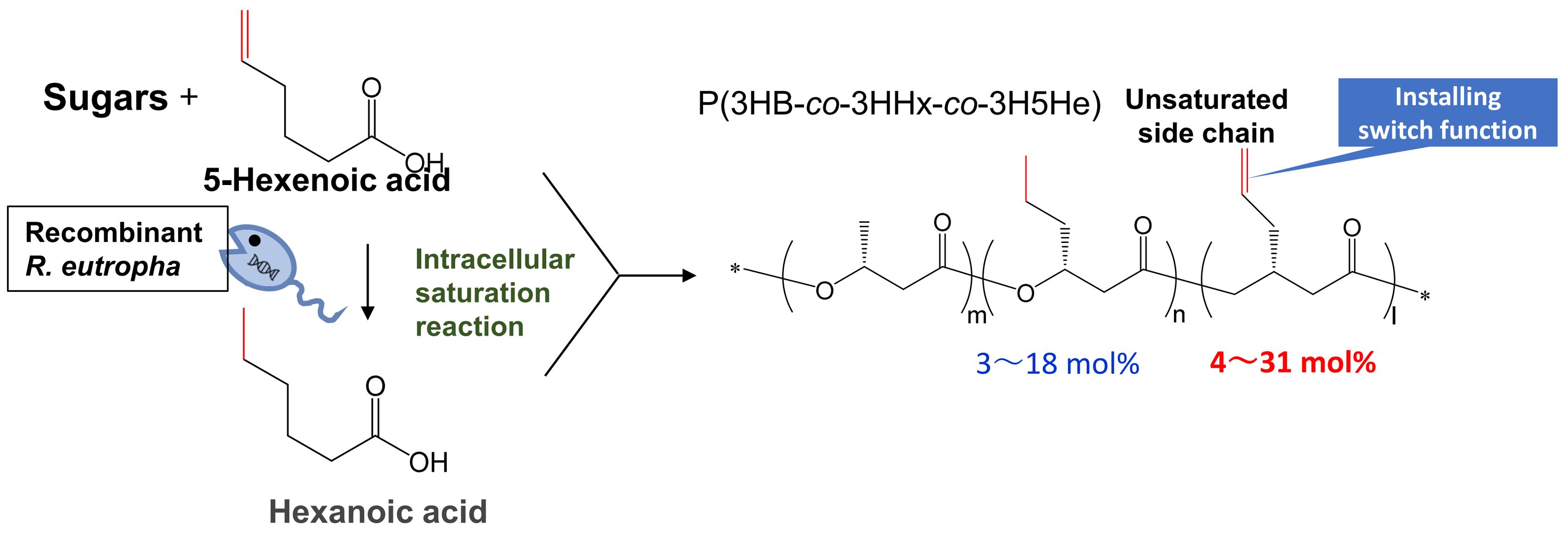
Contact: Gunma Univ Ken-ichi Kasuya (kkasuya@gunma-u.ac.jp)

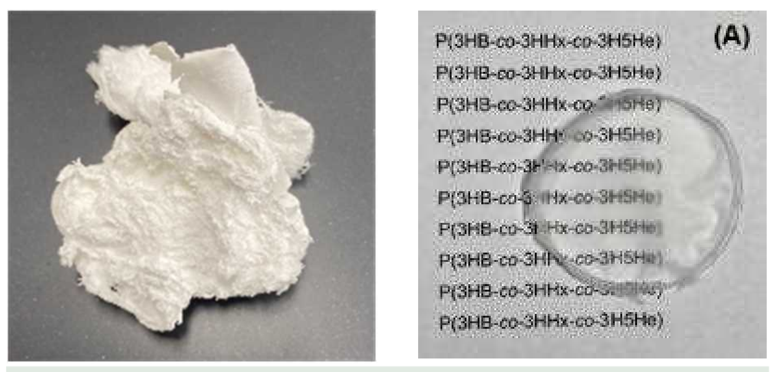
E1 E2 E3 E4 SI

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

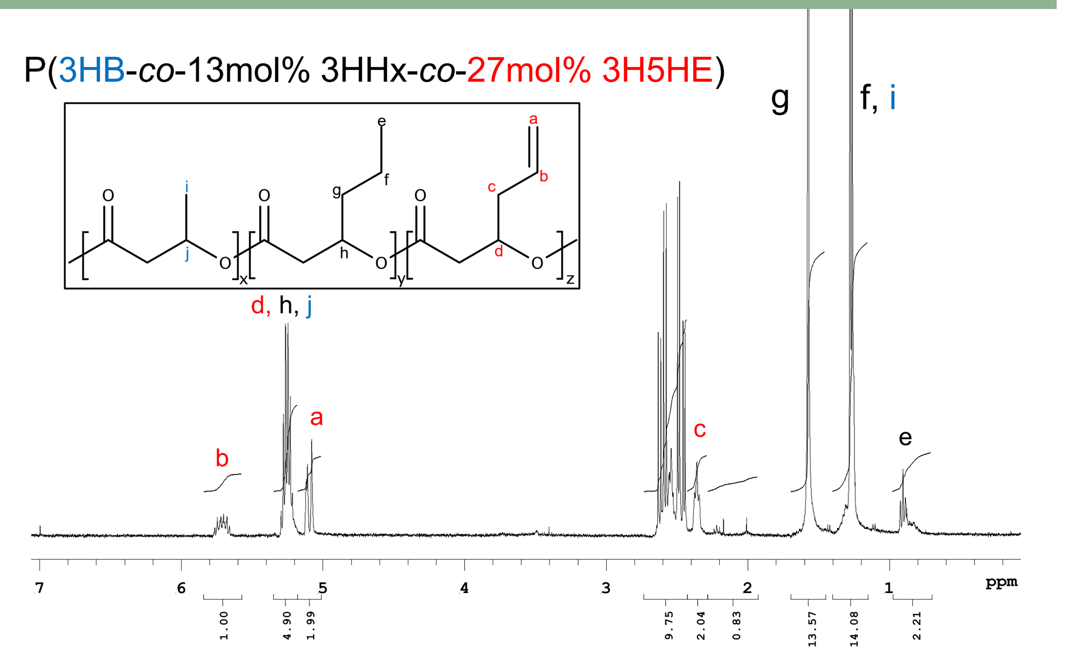


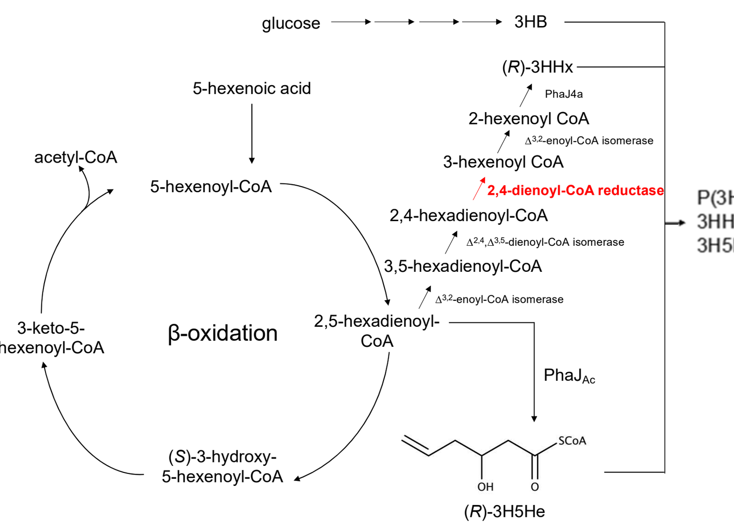
*In *Ralstonia eutropha*, the terminal double bonds of unsaturated fatty acids are easily reduced to saturated fatty acids.



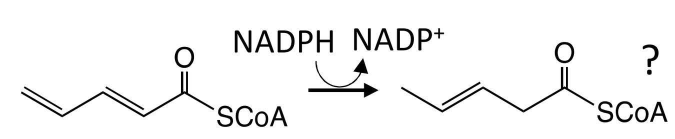


P(3HB-*co*-15.8% 3HHx-*co*-9.0 mol% 3H5He)
Mw: 28.9 x10[5], PDI: 2.4, Tg: -1.6°C, Tm: 119°C
Tensile strength: 14 MPa,
Elongation to break: 1,158%



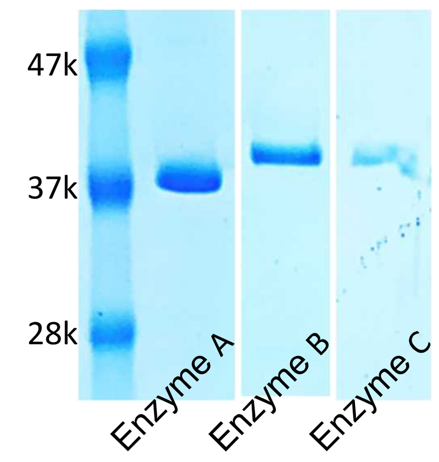


Identification of terminal unsaturated bond reductase



Triple gene knockout strain：
4-Pentenoic acid-added culture
→ Decline in PHA production

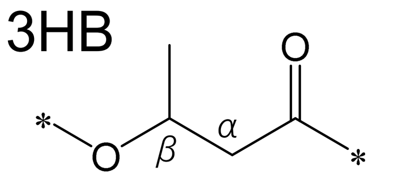
Recombinant enzyme by *E. coli* host



Toughness exceeding that of nylon 66（80 MJ/m3）

Conventional monomer

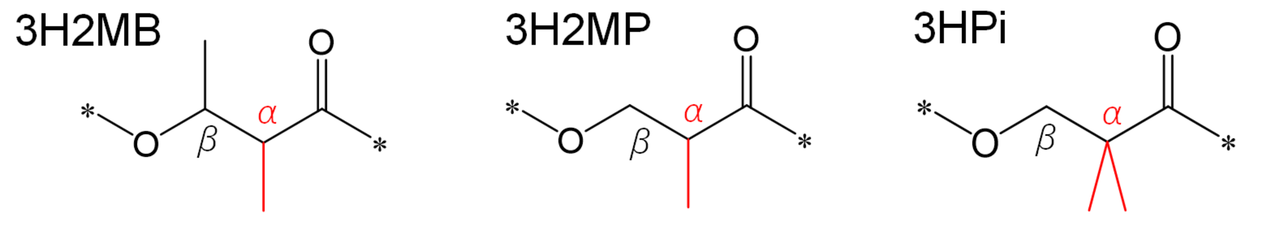
3HB



3-Hydroxybutanoic acid

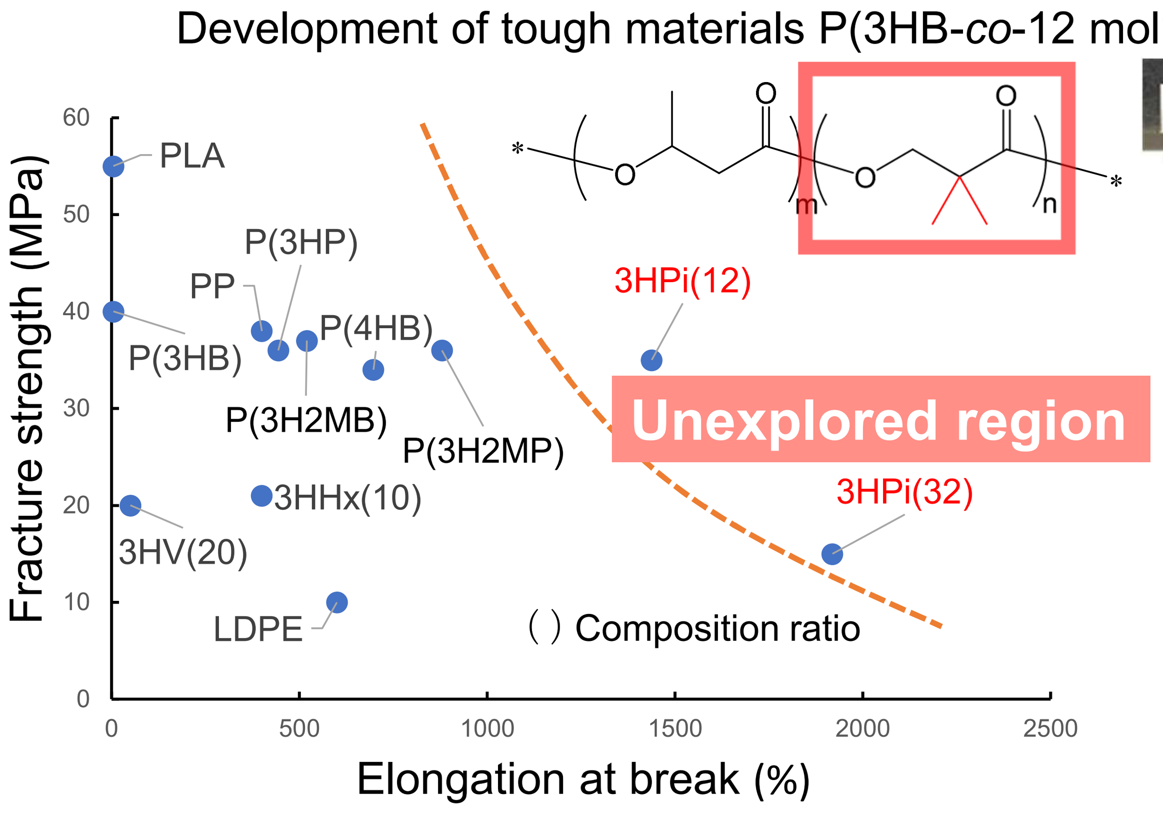
Methylated at α-position monomer

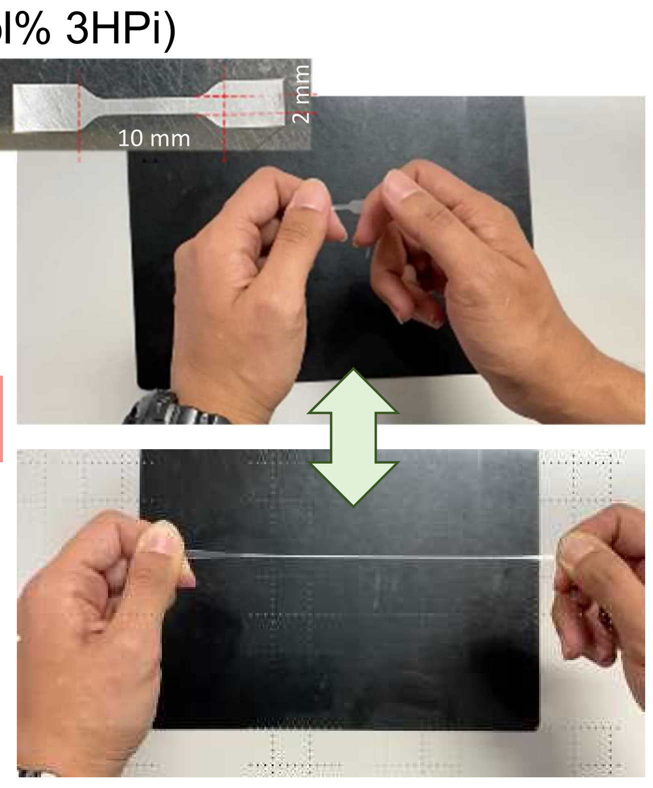
3H2MB 3H2MP 3HPi



3-Hydroxy-2-methyl butanoic acid / 3-Hydroxy-2-methyl propionic acid / 3-Hydroxypivalic acid

Development of tough materials P(3HB-*co*-12 mol% 3HPi)

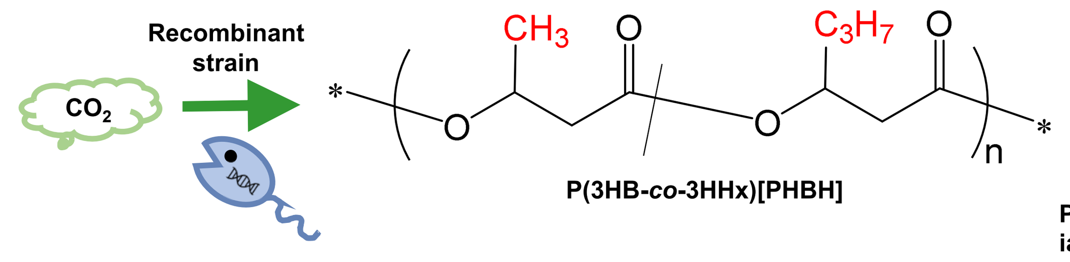


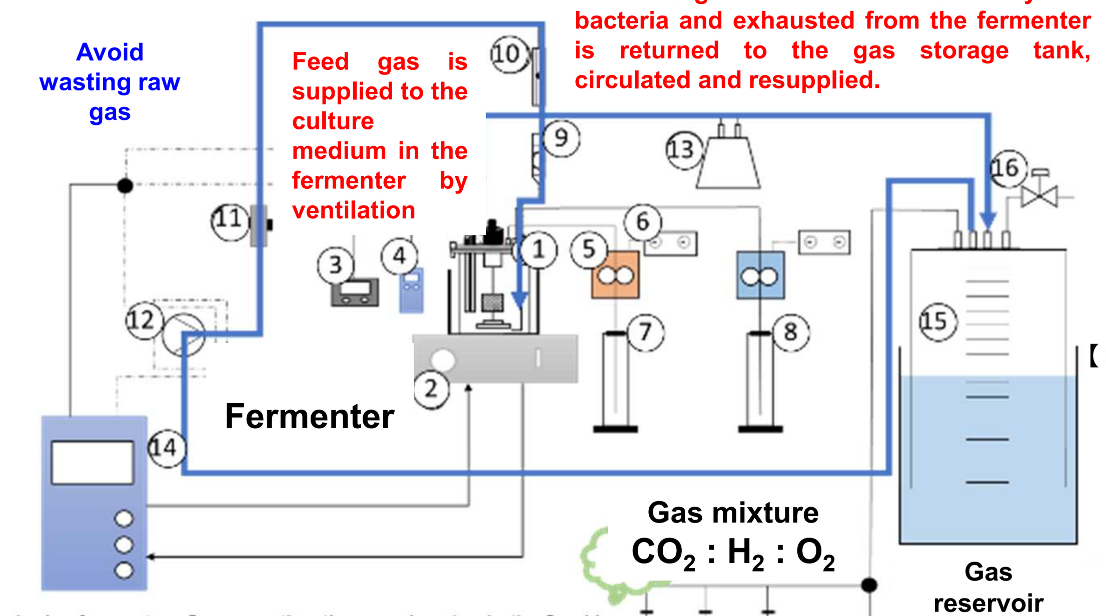


P(3HB-*co*-X)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	σ (MPa)	ε (%)	E (MPa)	Toughness (MJ/m^3)
12 mol% 3HPi	5	61	28	35	1438	306	306
32 mol% 3HPi	1	57	3	15	1919	219	150

Direct PHA conversion from CO_2

- Using H_2:O_2:CO_2 gas as a raw material substrate, genetically modified strains of *Ralstonia eutropha* (*C.necator*) were cultured, and a production test of a new polyester with excellent marine degradability is conducted.
- Clarifying the culture characteristics of recombinant strains and developing technology to efficiently produce polyester from CO_2.
- In particular, we will focus on the development of a culture method that enables the improvement of product concentration and speed, and the complete consumption of raw material gas.





1. Jar fermenter, 2. magnetic stirrer and water bath, 3. pH controller, 4. DO meter, 5. tube pump, 6. On-Off timer, 7. 7 w/w% ammonia water, 8. antifoam reagent, 9. sterile filter, 10. flow meter, 11. needle valve, 12. gas circulating pump, 13. drain trap, 14. temperature controller with circulating pump, 15. gas reservoir (water sealed gas holder), 16. gas sampling port, 17. manometer, 18. gas cylinders, 19. vacuum pump

Closed circulation gas culture system

Production of high PHBH concentration by iar culture of *C.necator* MF01/JAc *strain*

（2021）

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

Improved PHBH productivity

【Improvement of culture equipment and methods】

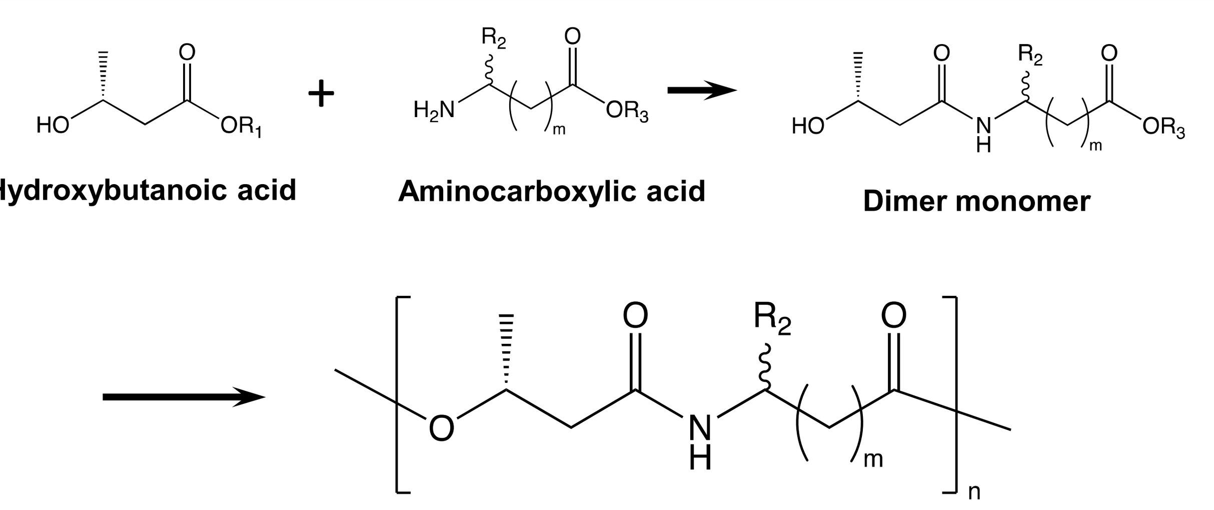
- Inorganic salts suitable for PHBH biosynthesis exploration of medium composition
- Improvement of fermenter agitation performance (raw material gas dissolution rate)
- Maintains low concentrations of inorganic nutrients
- Mass flow controller continuously replenishes feedstock gas with constant composition

（2023）

Dry cell (g/L)	Culture time(h)	Productivity (g/L/h)
98.2	142.5	0.689
PHBH (g/L)	**3HB (mol%)**	**3HHx (mol%)**
81.5	86.2	13.8

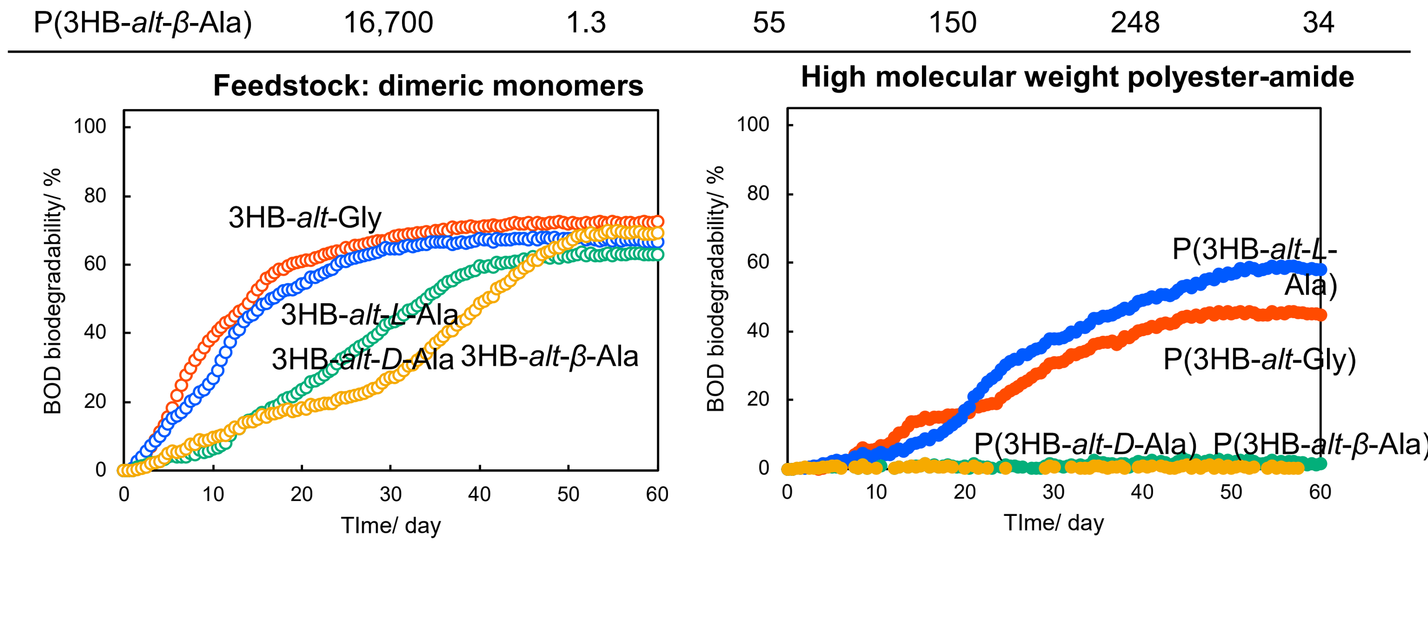
World Record: High polyester production from CO_2 including P(3HB)

Development of biodegradable polyamide

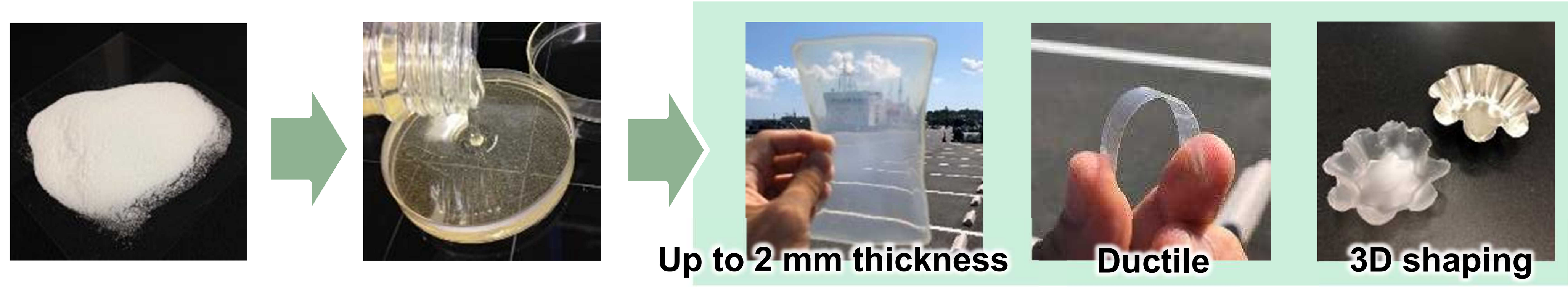


Ester amide alternating copolymer

	M_n / g·mol[-1]	M_w/M_n	T_g / °C	T_m / °C	$T_{d5\%}$ / °C	X_c / %
P(3HB-*alt*-Gly)	14,100	1.6	80	162	245	40
P(3HB-*alt*-*L*-Ala)	12,400	1.2	80	n.d.	241	36
P(3HB-*alt*-*D*-Ala)	5,300	1.3	85	n.d.	242	42
P(3HB-*alt*-*β*-Ala)	16,700	1.3	55	150	248	34



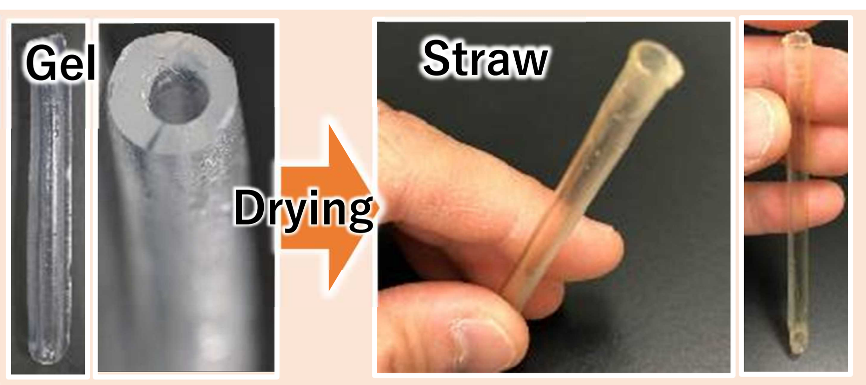
Biodegradable plastics from polysaccharides



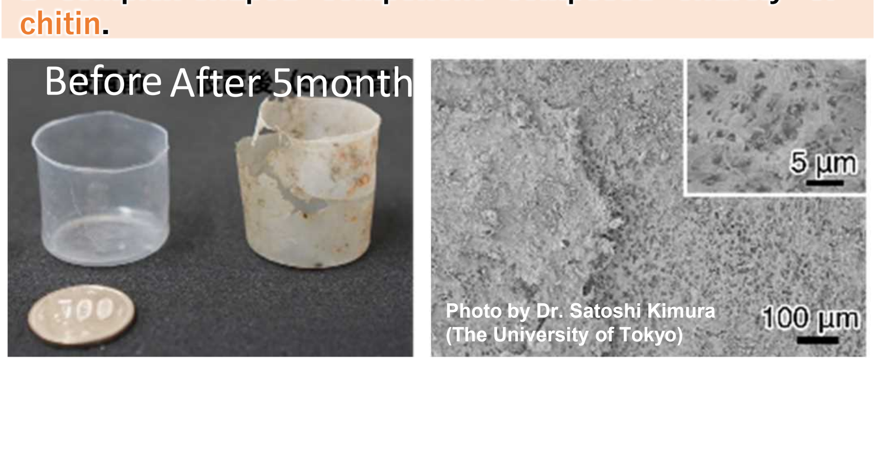
Dissolving, coagulating, and drying cellulose gives transparent paperboard. PCT/JP2020/039874

Compositionally identical with paper but more functional.

- **Molded into a cup shape, which is the expected shape for actual use.**
- **Demonstrated that the cup-shaped cellulose material biodegrades by half in less than six months at the bottom of the deep sea.**
- **Large numbers of degrading microorganisms were observed perforating on the surface of the material.**



The brittleness of the material, which had been a problem, was overcome by improving molding and processing techniques, and we succeeded in preparing a complex-shaped component composed entirely of chitin.



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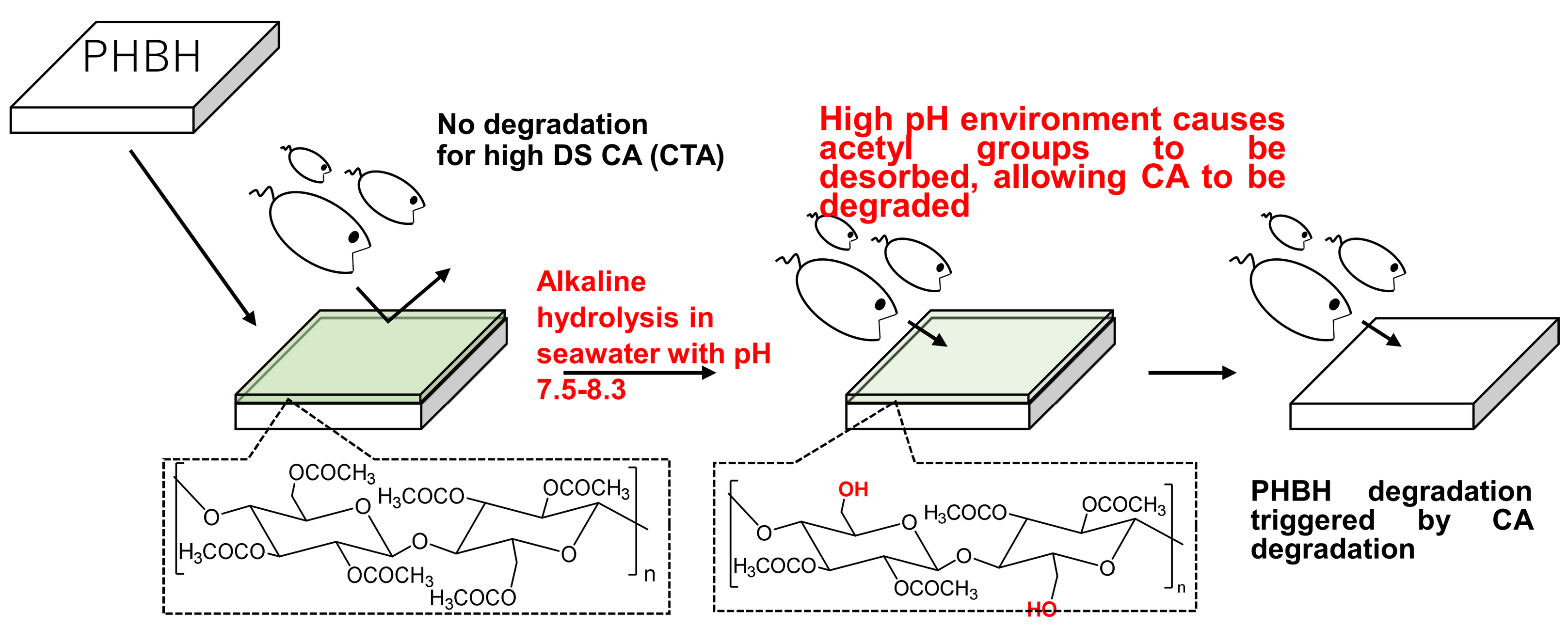
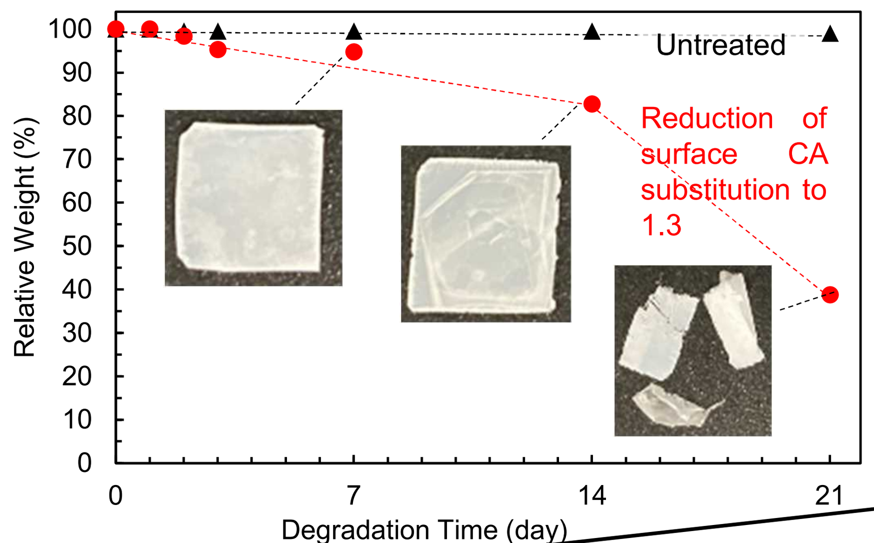
E1 E2 E3 E4 SI

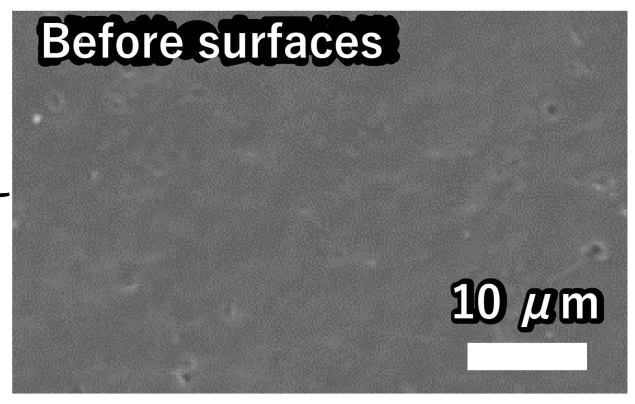
Switching triggered by difference in pH

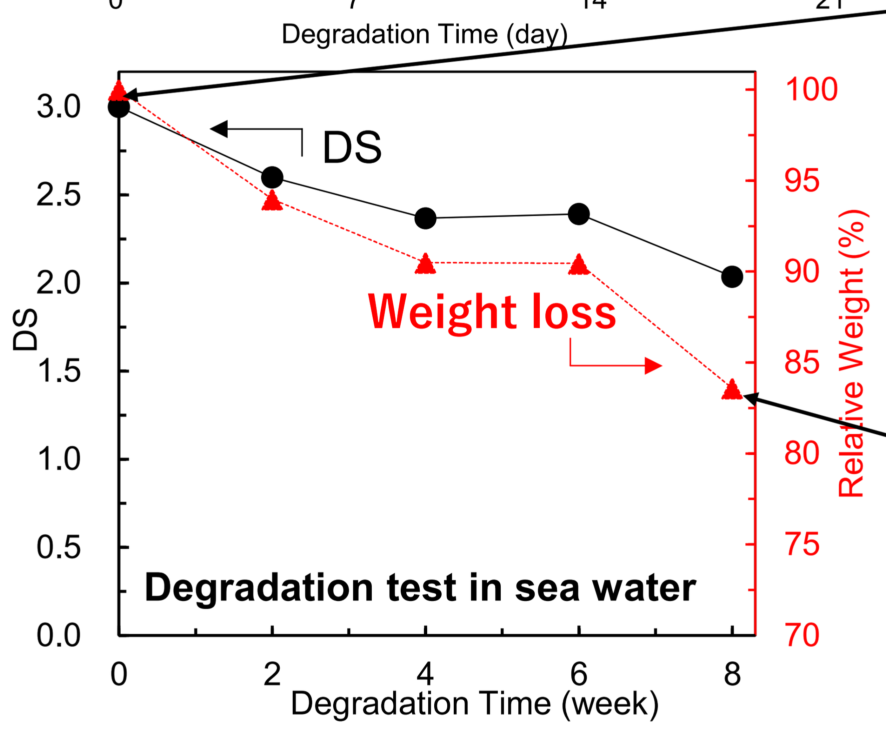
Cellulose triacetate (CTA) coating

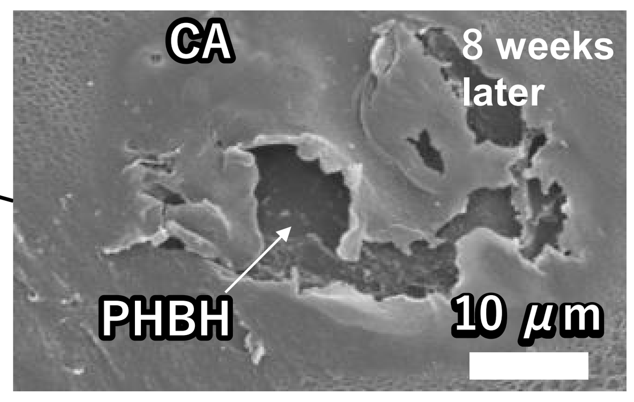


Enzymatic hydrolysis cellulase and PHB depolymerase



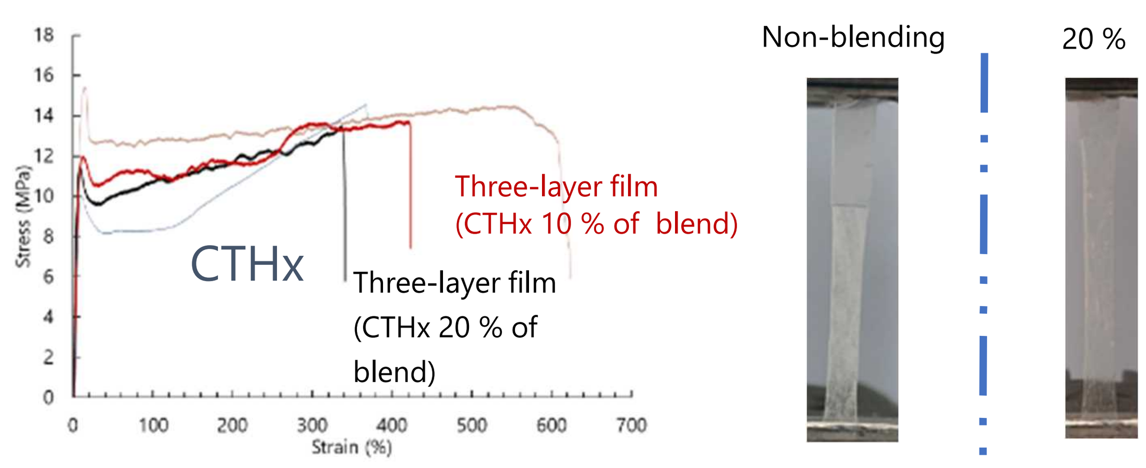






Degradation of CA exposes PHBH

Succeeded in improving interlaminar adhesion

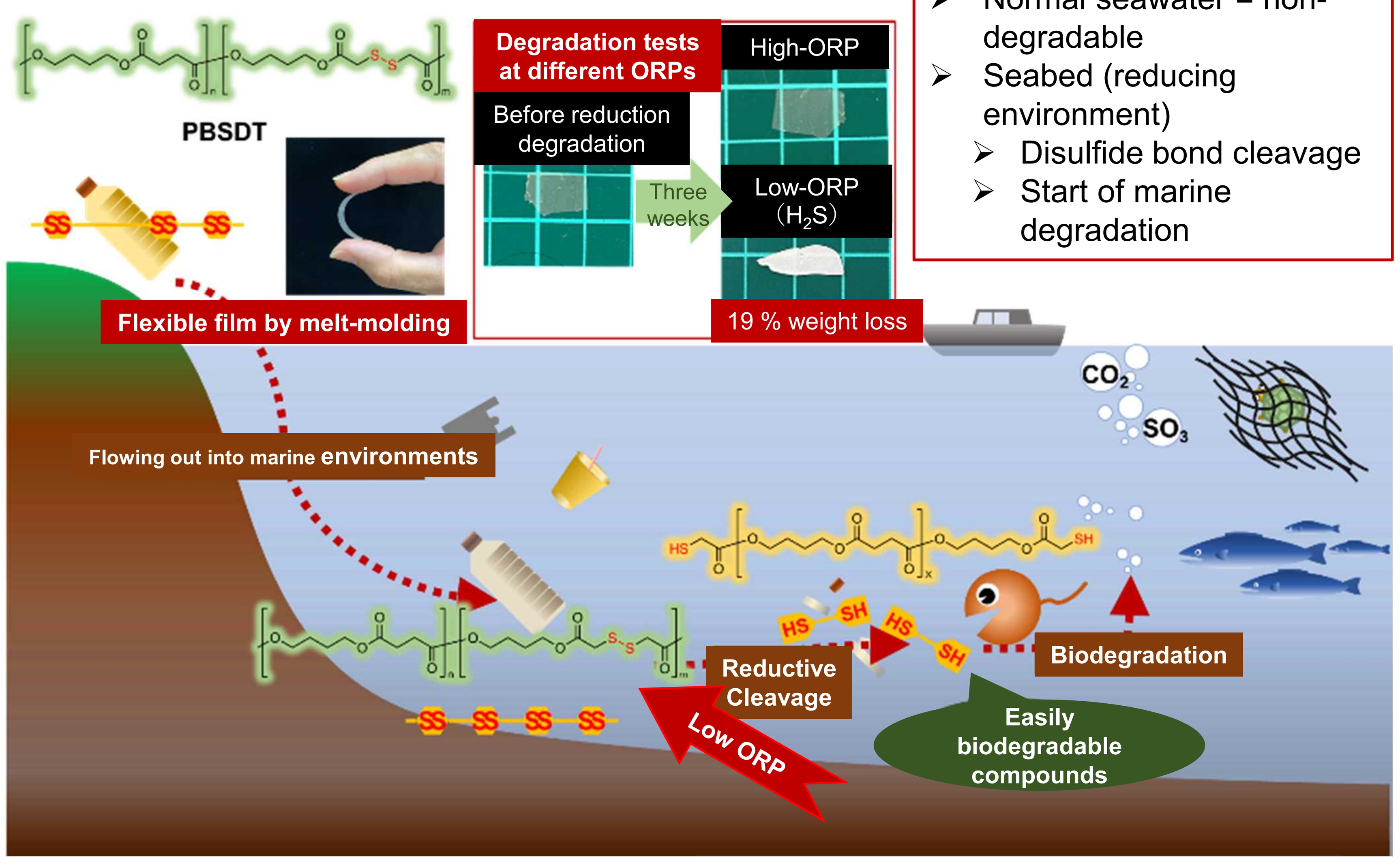


Base resin properties are expressed through improved adhesion

Switching triggered by difference in ORP

Biodegradability control by low oxidation-reduction potential (ORP) in marine environments

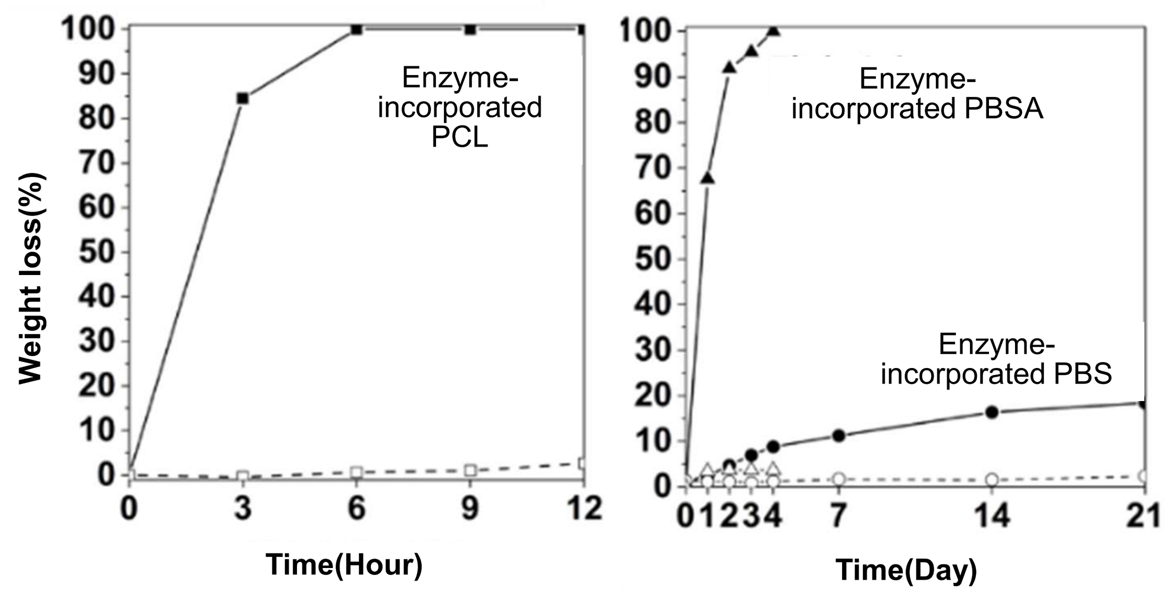
- PBSDT Physical Properties
  - PBSA analogues
  - Can be formed into films
  - Can be synthesized from commercial reagents
- Normal seawater = non-degradable
- Seabed (reducing environment)
  - Disulfide bond cleavage
  - Start of marine degradation

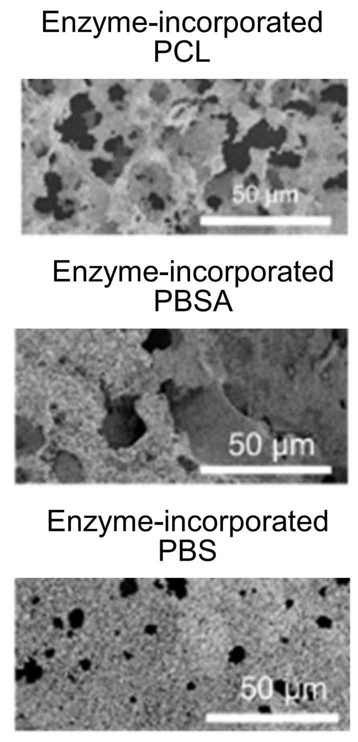


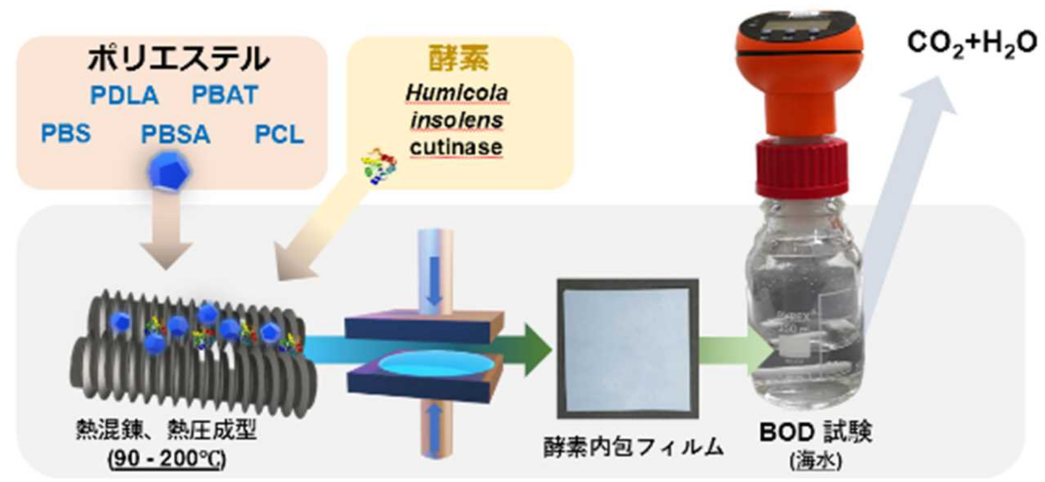
Switching triggered by wear

Switching triggered by wear (Enzyme)

Degradation of CaLB lipase-incorporated PCL, PBS, and PBSA in water



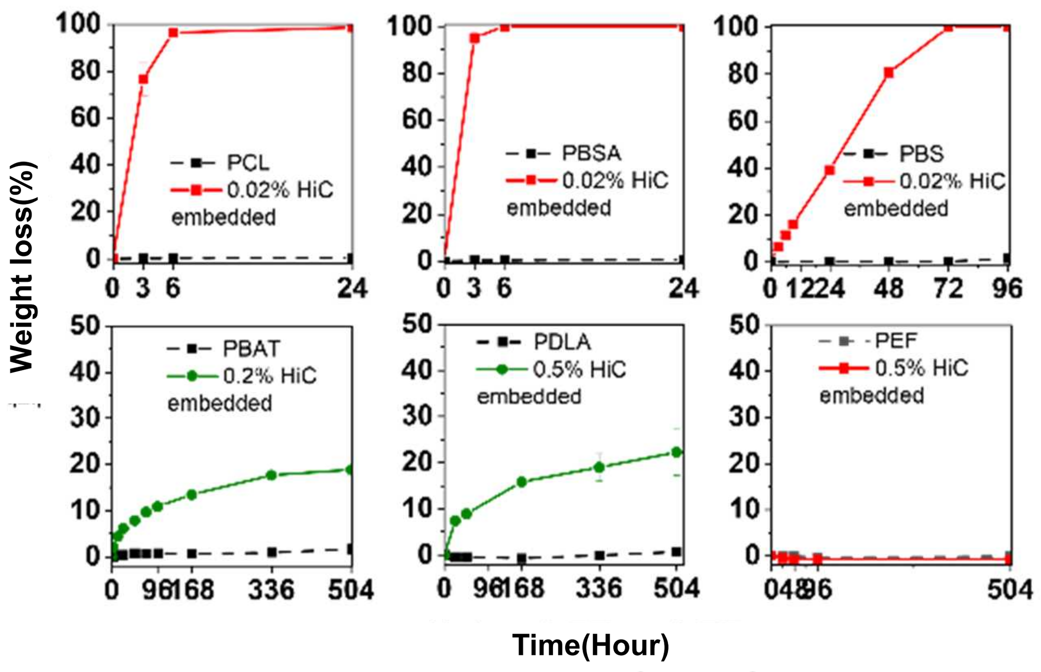




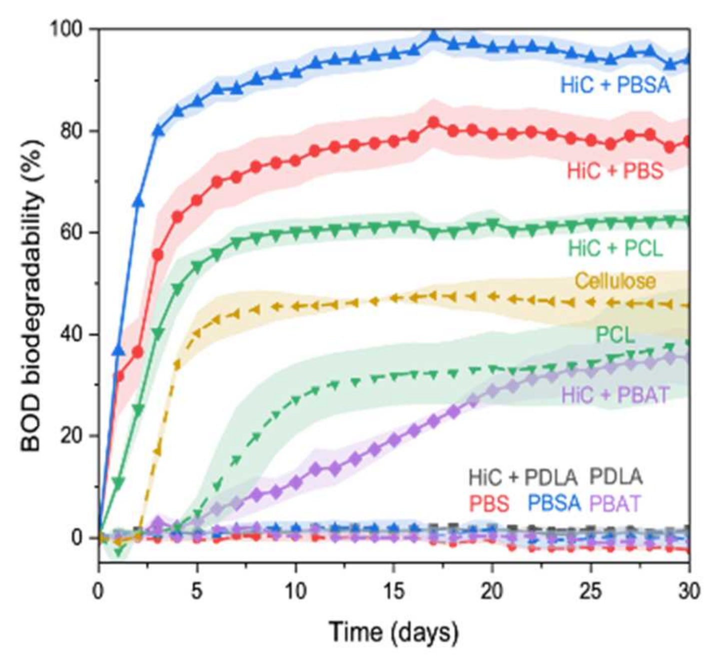
- PCL, PBS, and PBSA also succeeded in making enzyme-embedded plastics by melt blending.
- PCL completely degraded in 6 hours and PBSA in 3 days.
- PBS degrades 20% in 21 days.

Development of enzyme (lipase) embedded biodegradable plastics

Degradation of biodegradable plastics melt-mixed with cutinases in a buffer



BOD biodegradability curves of various biodegradable plastics melt-mixed with cutinases in seawater of Tokyo Bay

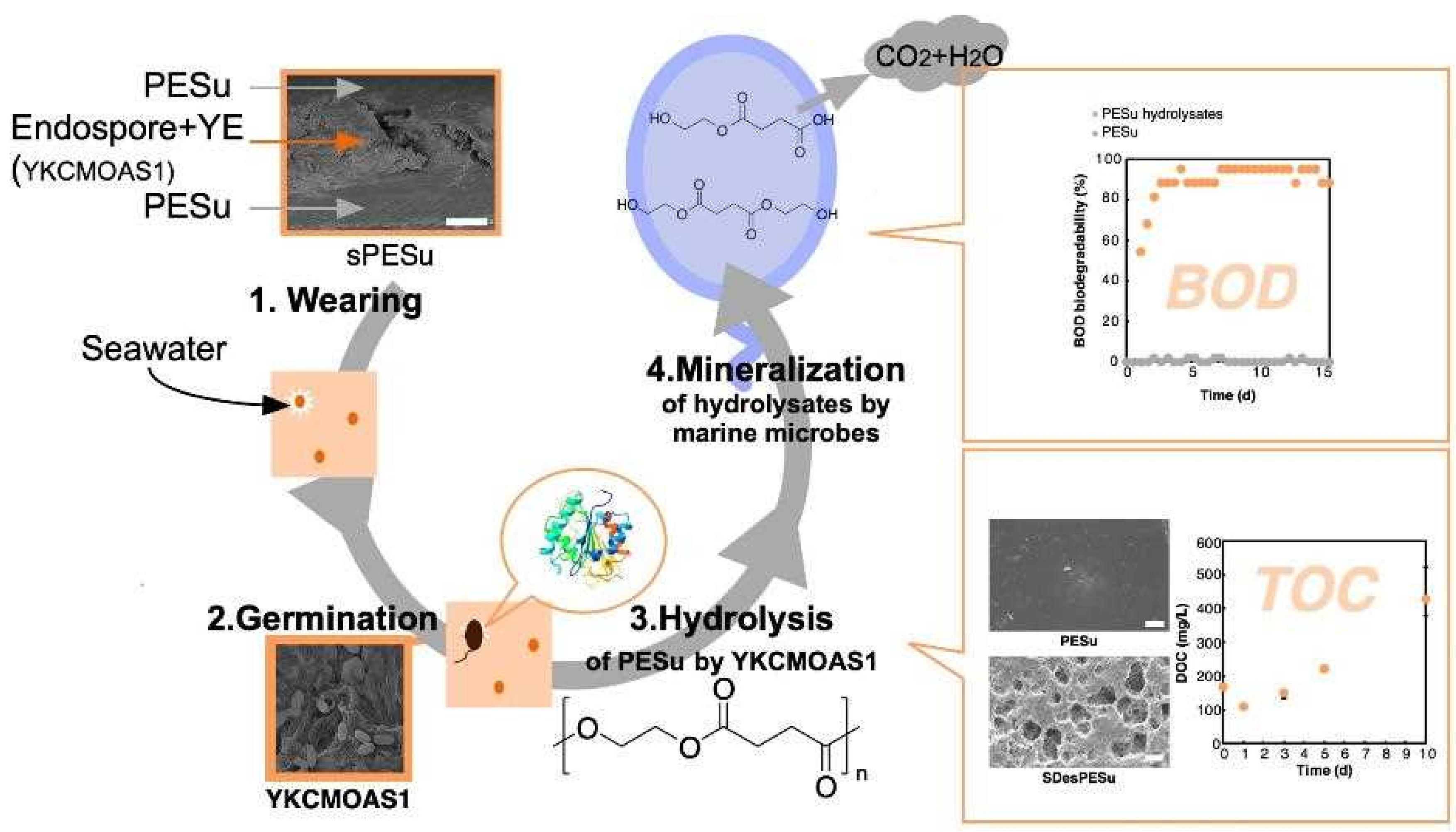


Successfully reduced degradation time

Normal biodegradable plastics hardly degrade in seawater, but enzymatic encapsulation proved to be more degradable than cellulose.

Switching triggered by wear (Endospore)

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



PESu degraded in the marine environment by spore-forming bacteria was eventually mineralized in the marine environment.

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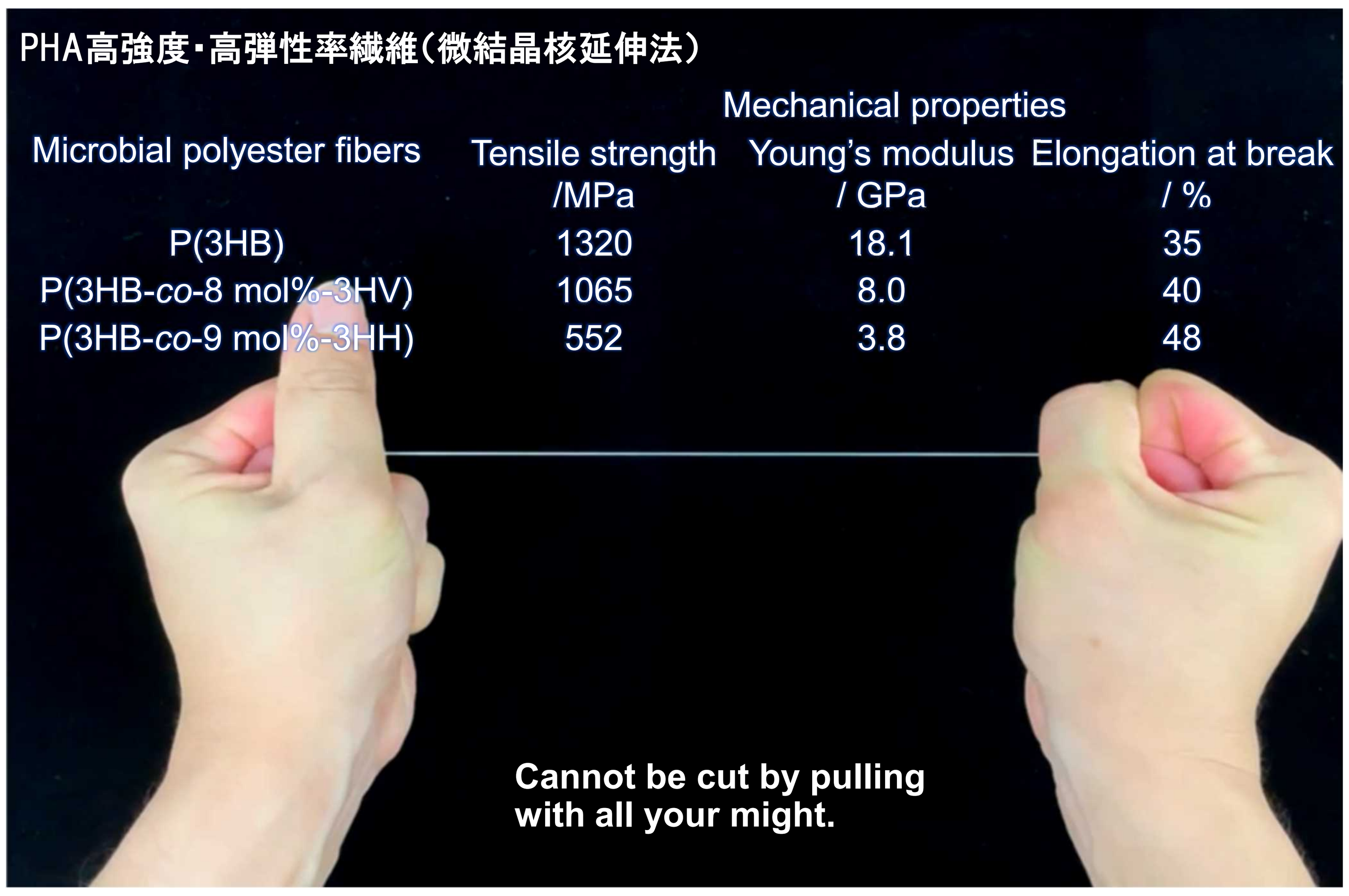
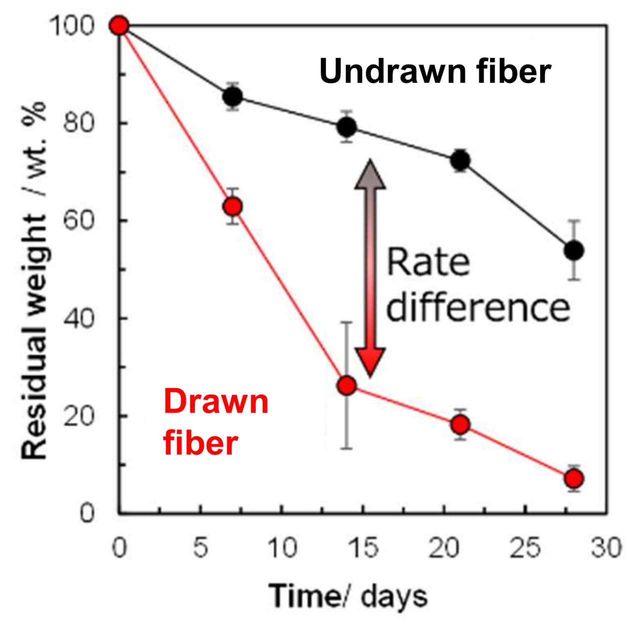
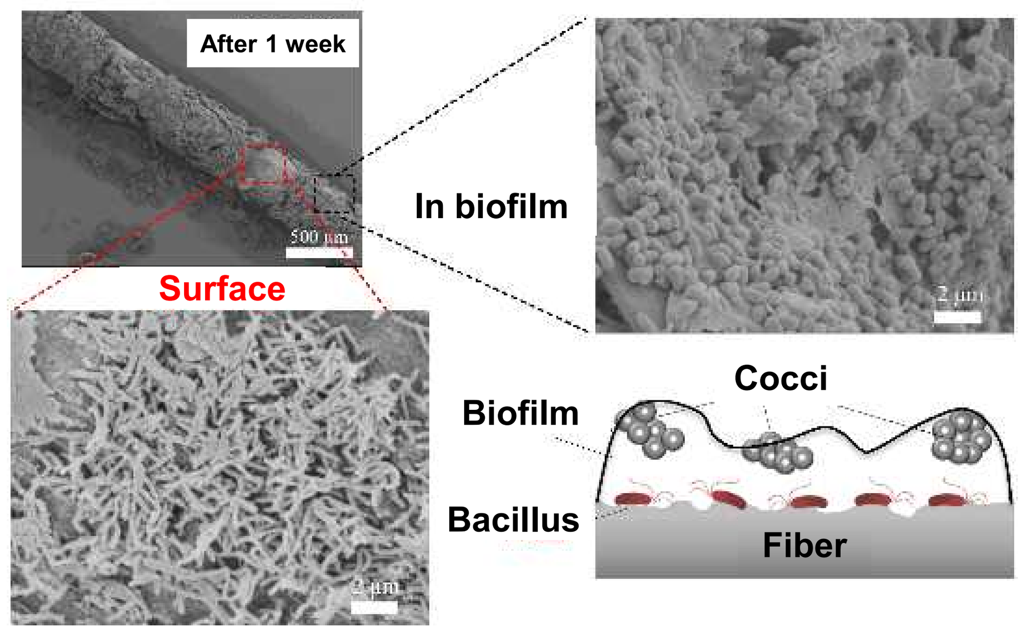
E1 E2 E3 E4 SI

Biodegradation rate factors from materials science





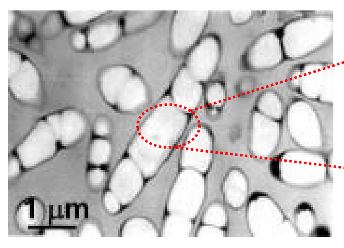
Biodegradation of fiber

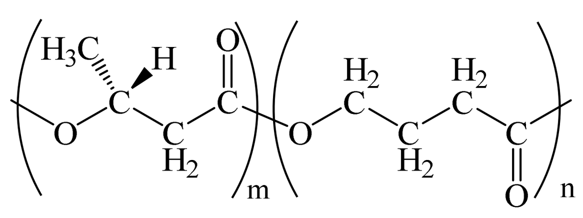


Bacillus on the fiber surface may degrade fiber using the enzyme.

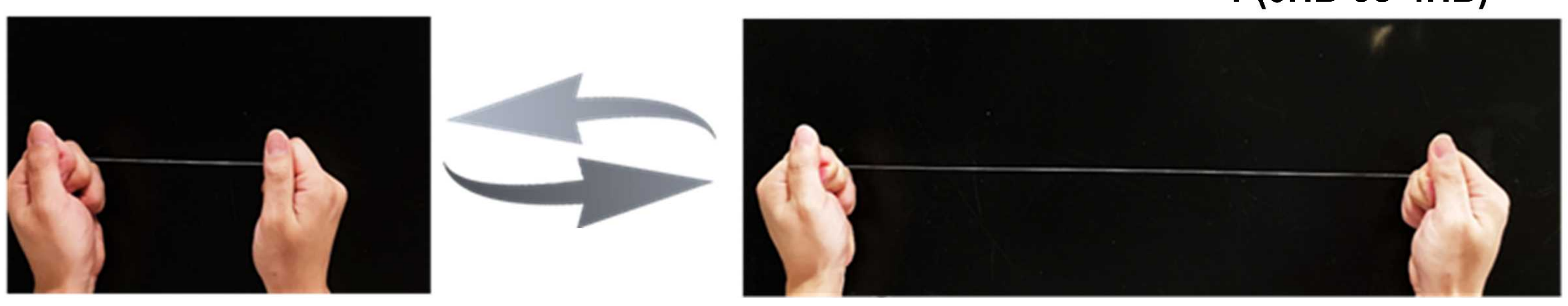
- Degradation rate can be controlled by drawing ratio.
- Degradation rate is related to crystalline morphology.

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



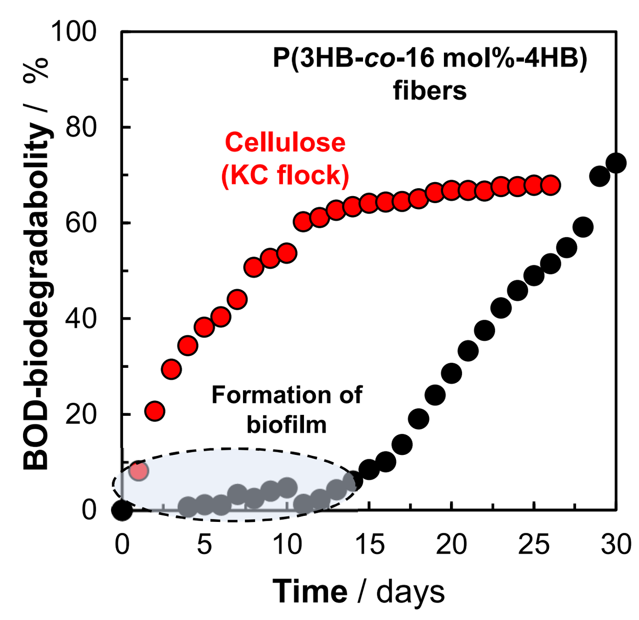


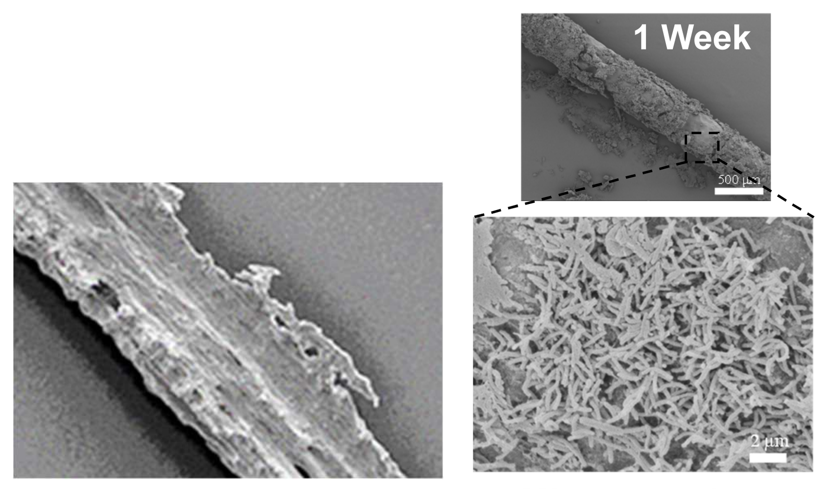
P(3HB-*co*-4HB)



Highly stretchable biodegradable fibers

BOD biodegradability in seawater

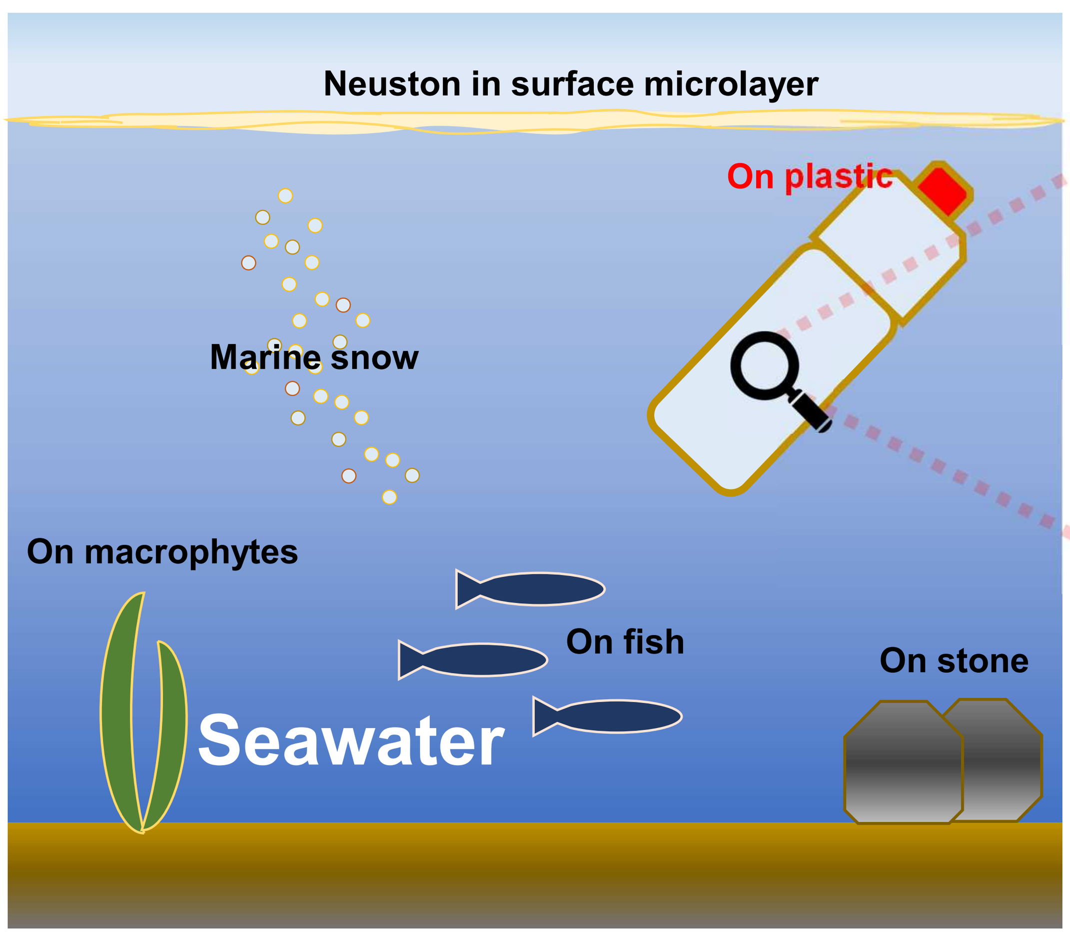


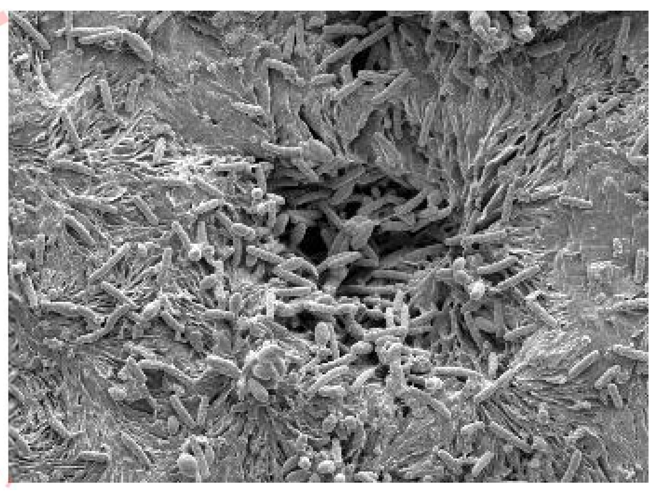


SEM image of the fiber during degradation

Microorganisms on fiber surface

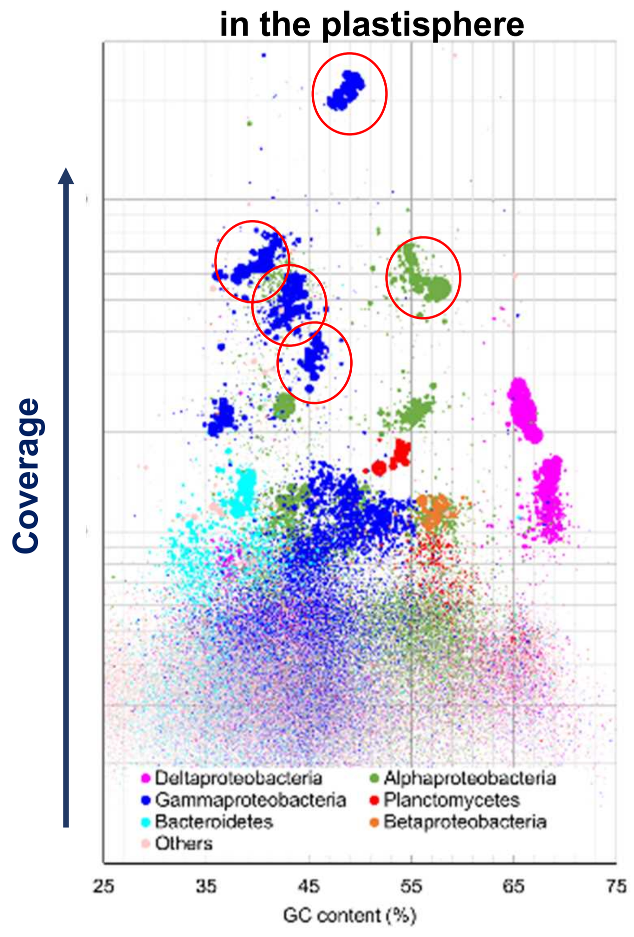
Plastisphere: Microbial flora formed on plastic surface





SEM image of plastisphere formed on the biodegradable plastic surface.

Metagenome analysis

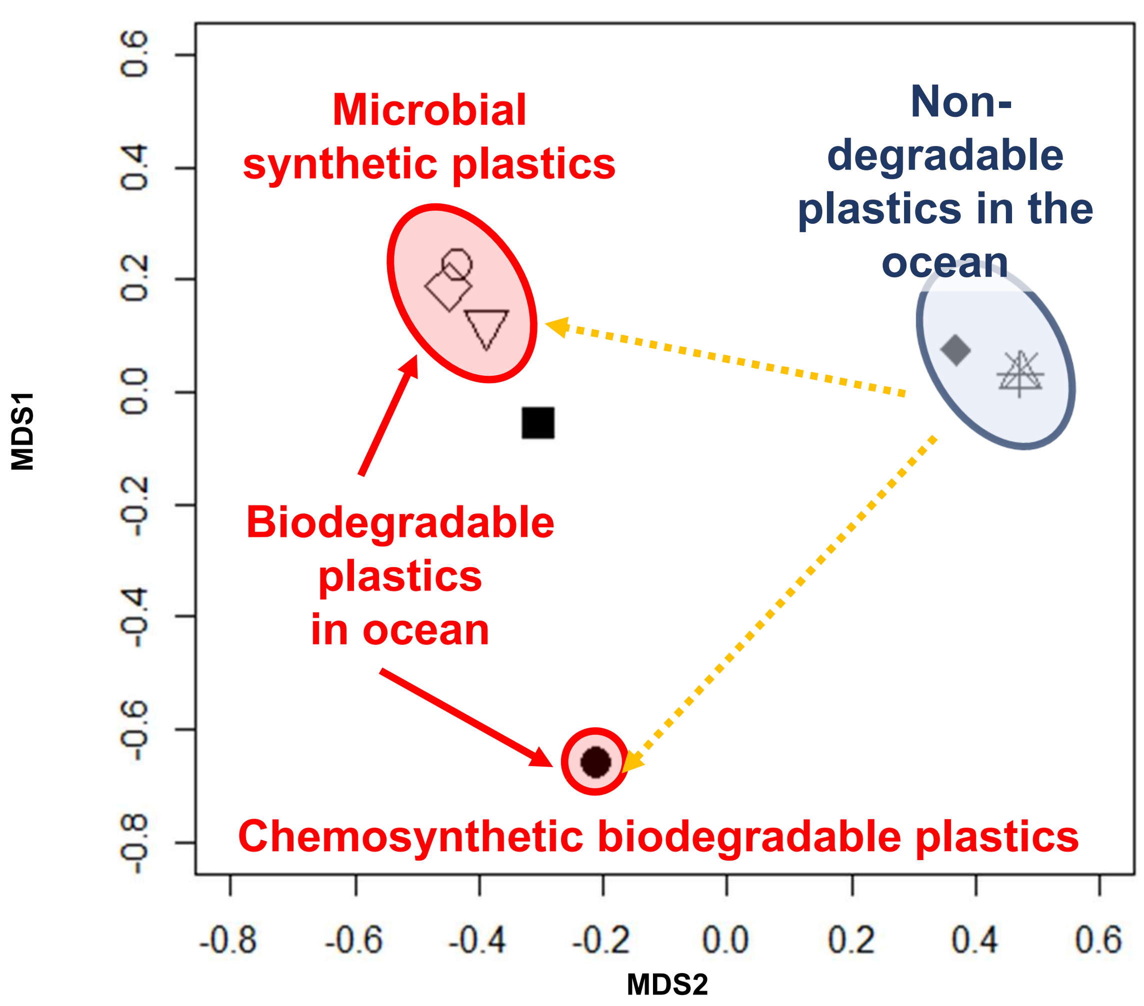


Microbes with high abundance = Genome information of microbes involved in the biodegradation.

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

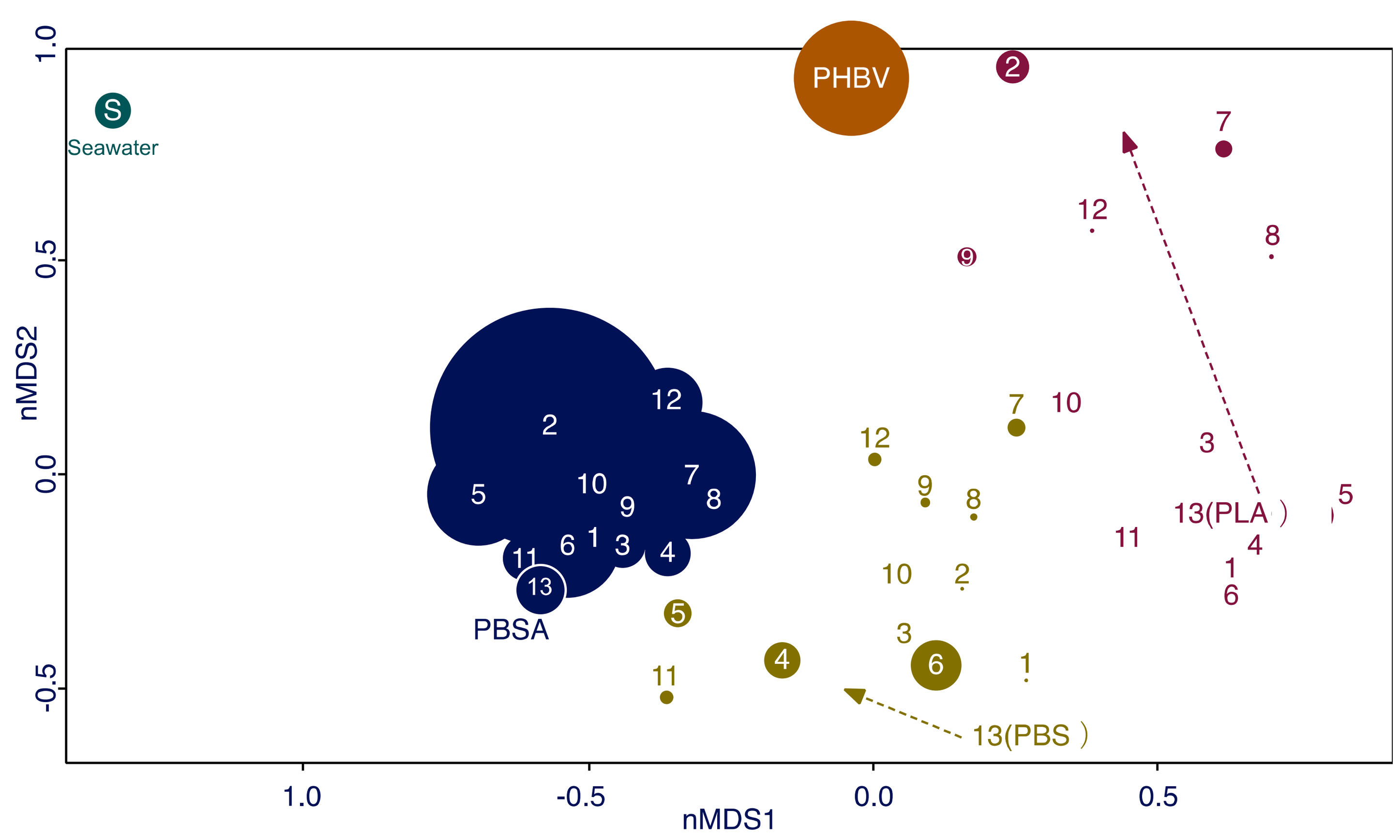
Also investigate the plastisphere in non-oceanic environments.

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-16-5E

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, Tokyo Tech

Contact: Gunma Univ Ken-ichi Kasuya (kkasuya@gunma-u.ac.jp)

E1 E2 E3 **E4** SI

Biodegradation tests of novel materials

<Types of marine biodegradation tests>

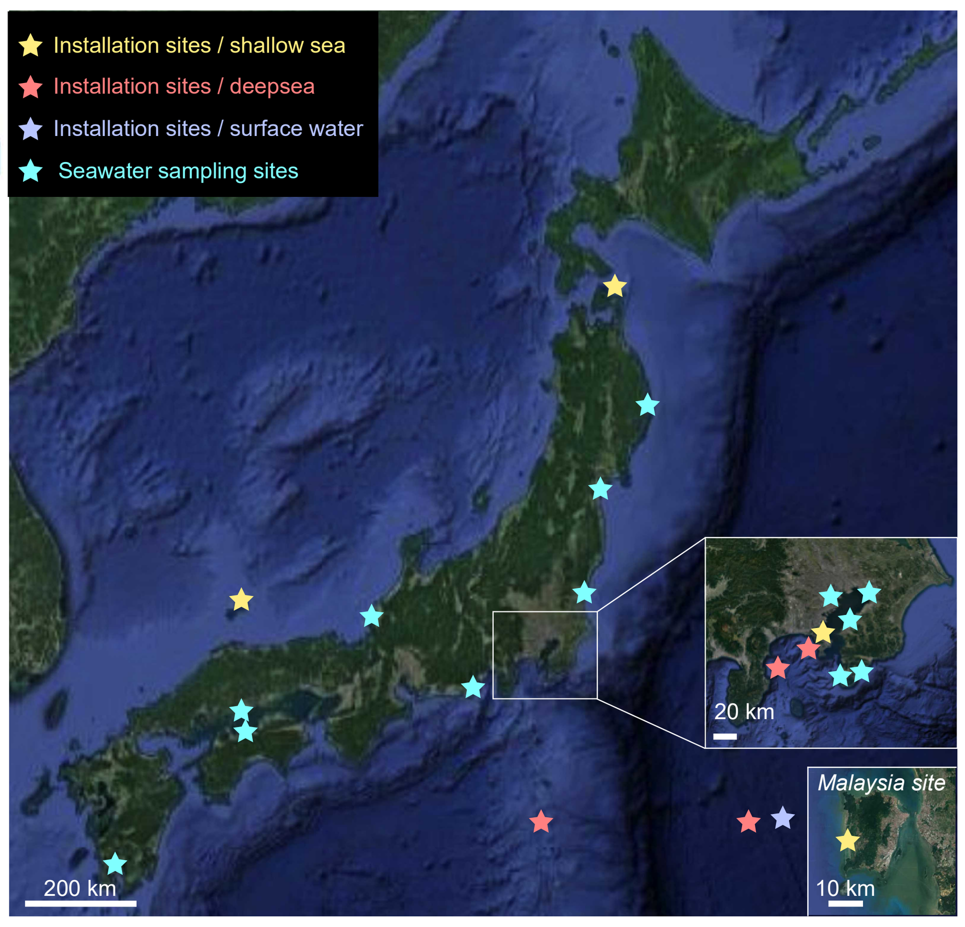
- *In vitro*
  - Normal pressure (water tank, BOD)
  - Pressurized (pressurized vessel)
- *In situ*
  - Shallow water
  - Deepsea water
  - Pelagic surface environment

Sites for degradation test and seawater sampling site

Deepsea	4	★
Shallow water	4	★
Pelagic surface	1	★
Mangrove	1	★
Seawater sampling	13	★★

(One of them is collecting deepsea water at any time.)

Degradation testing was conducted in a variety of marine environments.



Joint implementation with NEDO KUNIOKA PJ "Establishment of Evaluation Methods for Marine Biodegradability"

- Biodegradation assessments in the Deep Sea and Information Exchanges
  - Conducting experiments in the deep sea using the Shinkai 6500
  - Promotion committee meetings and joint workshops held (4 times / year)

Joint implementation with NEDO Moonshot ITO PJ

- Biodegradation assessments, Publicity Activities in Southeast Asia, and Information Exchanges
  - Degradation testing of marine biodegradable plastics (Malaysia, Thailand, and Indonesia)
  - Workshops in Thailand and Malaysia (Fall 2024 scheduled)
  - Promotion committee meetings and joint workshops held (4 times / year))

In situ biodegradation tests

Between 2020 and 2023, seven cruises were conducted to test the biodegradability of newly developed materials on the deepsea floor.

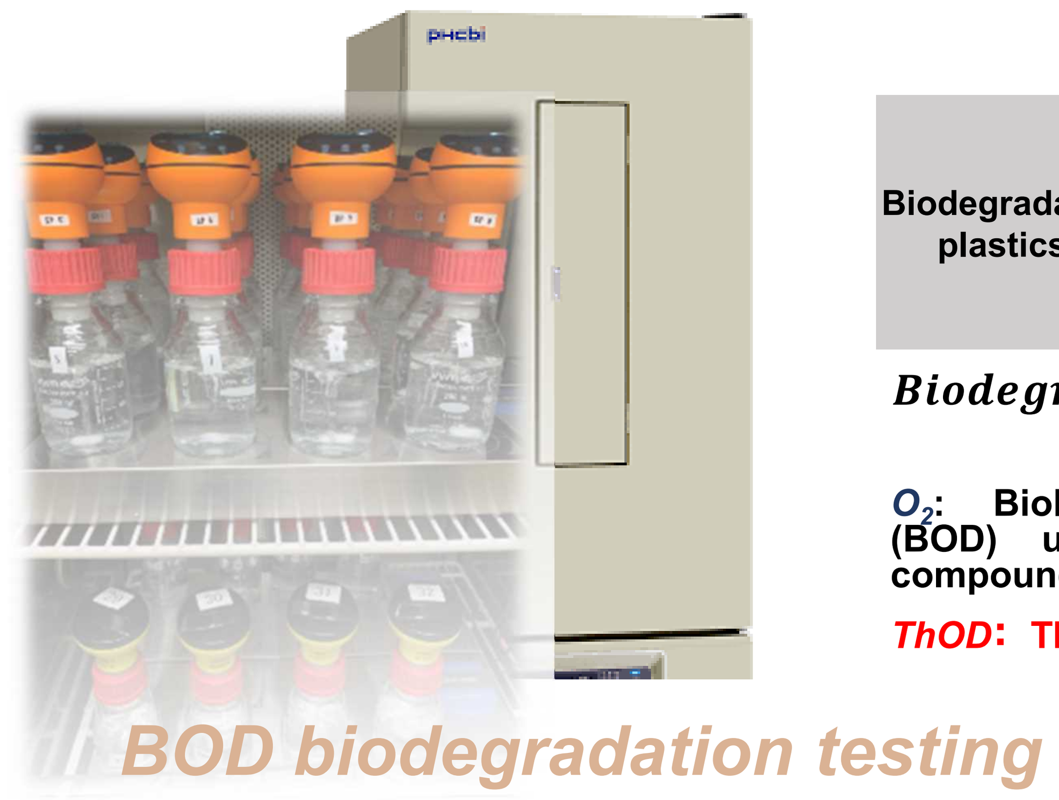
This project is unique in that it tests biodegradability in situ on the deepsea floor, where large amounts of plastic debris are deposited.

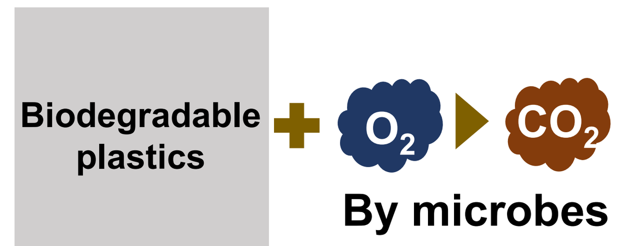
Marine Center, Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center

Oki Coastal Lab., Shimane University

Sample installation at Mutsu Lab, Aomori Pref

In vitro biodegradation tests

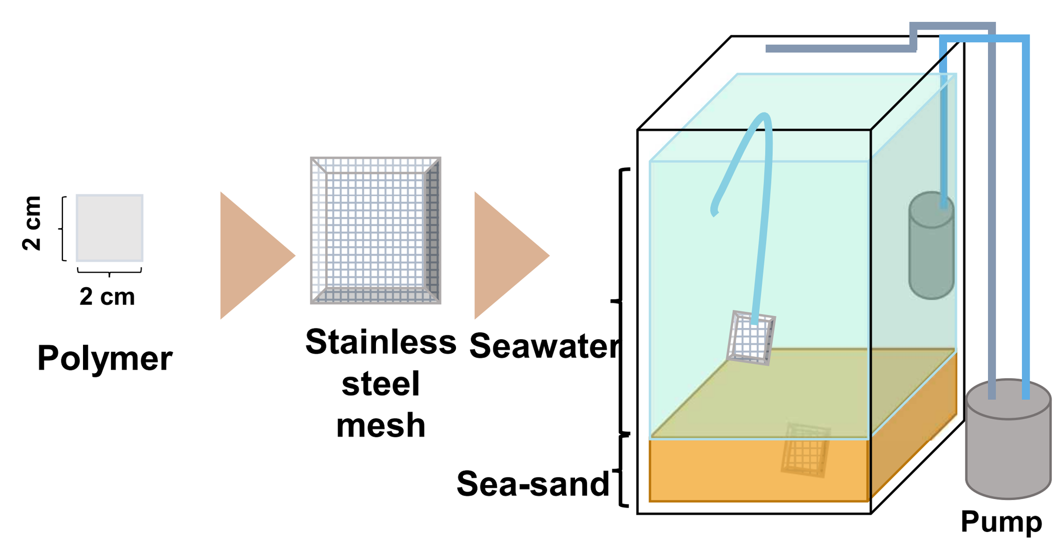




$$Biodegradability\% = \frac{O_2}{ThOD} \times 100$$

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

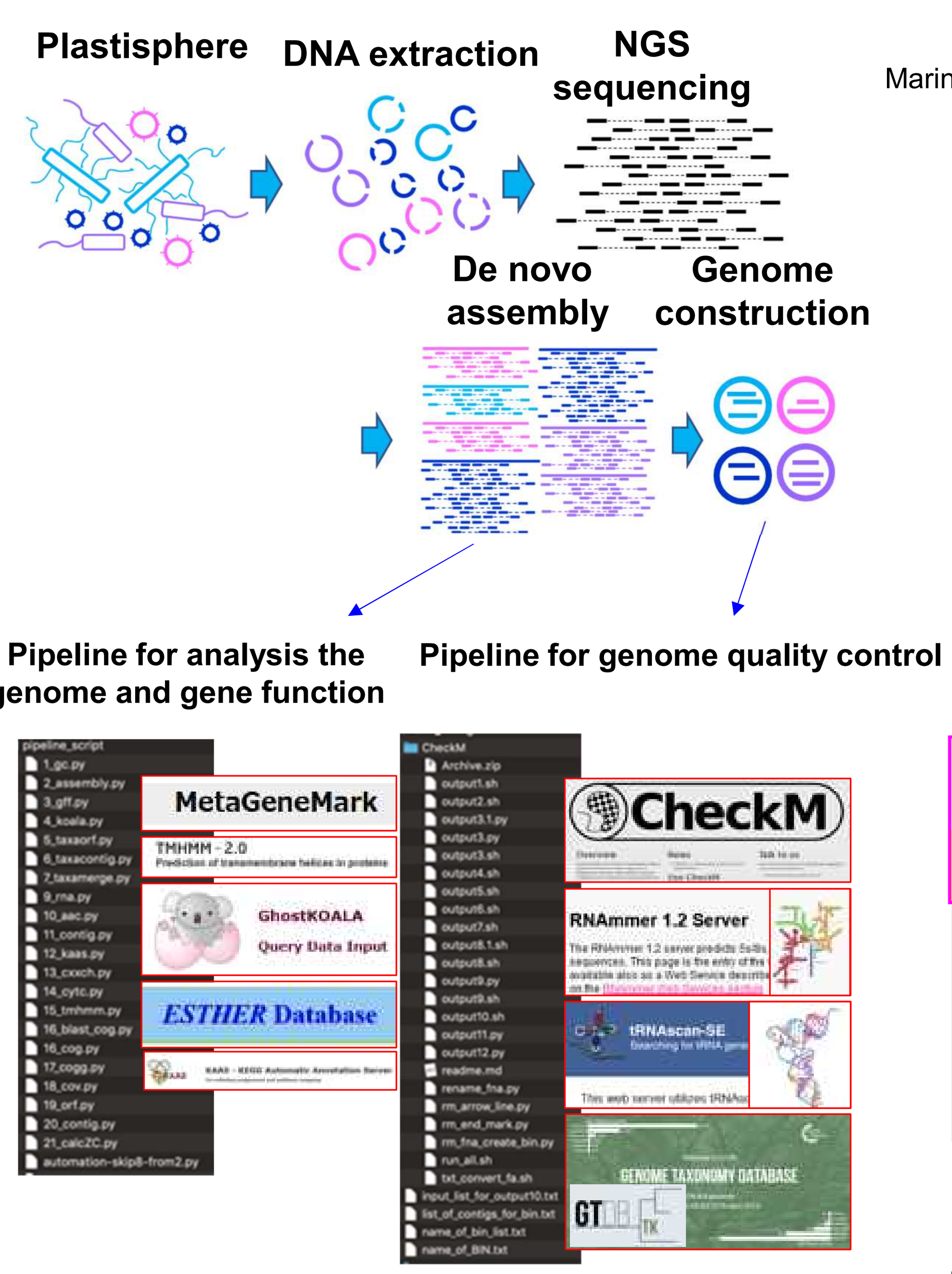
ThOD: Theoretical oxygen demand



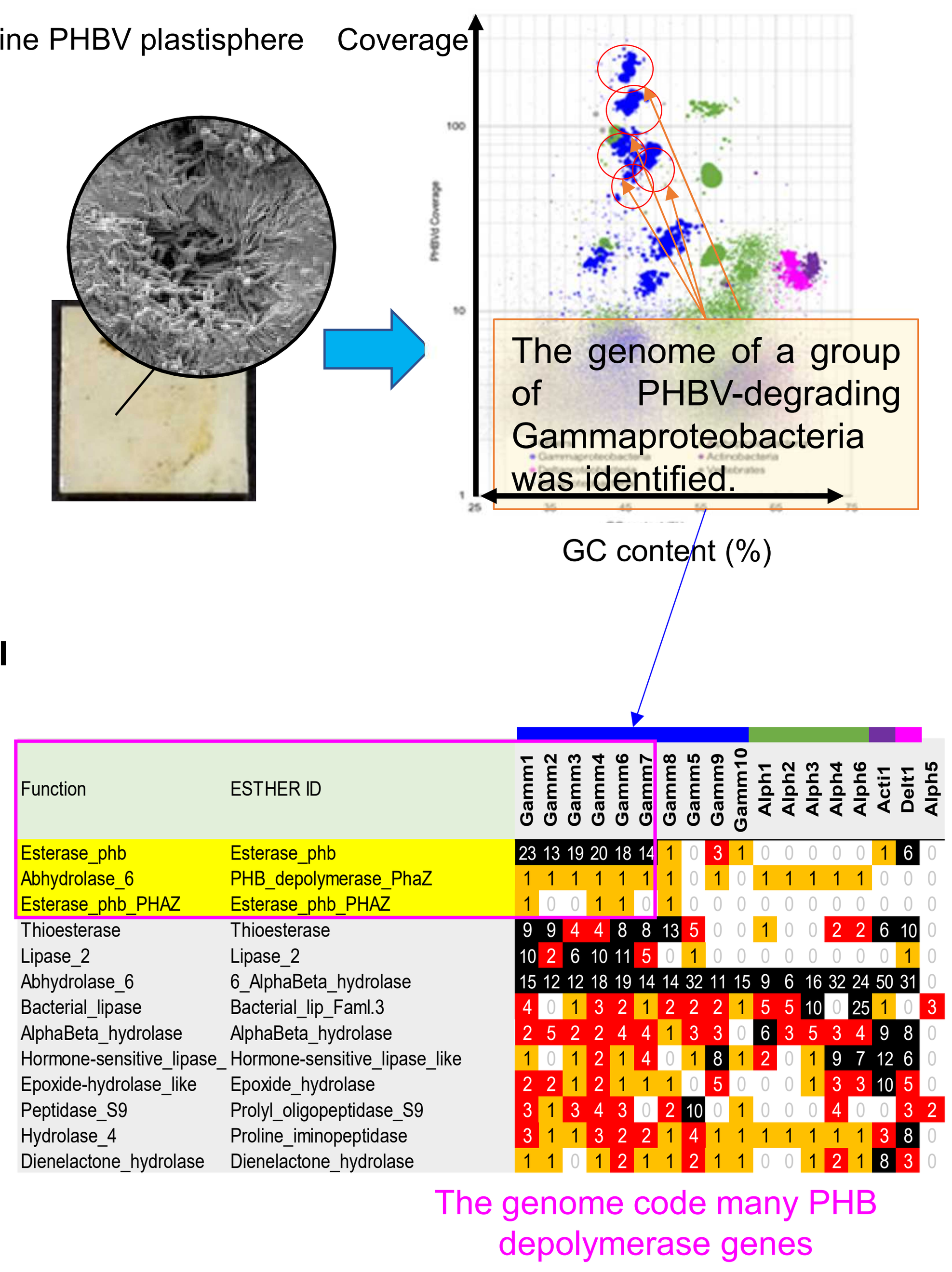
- Weight loss
- Morphology of surface
- Mechanical properties
- Plastisphere analysis

Tank experiment

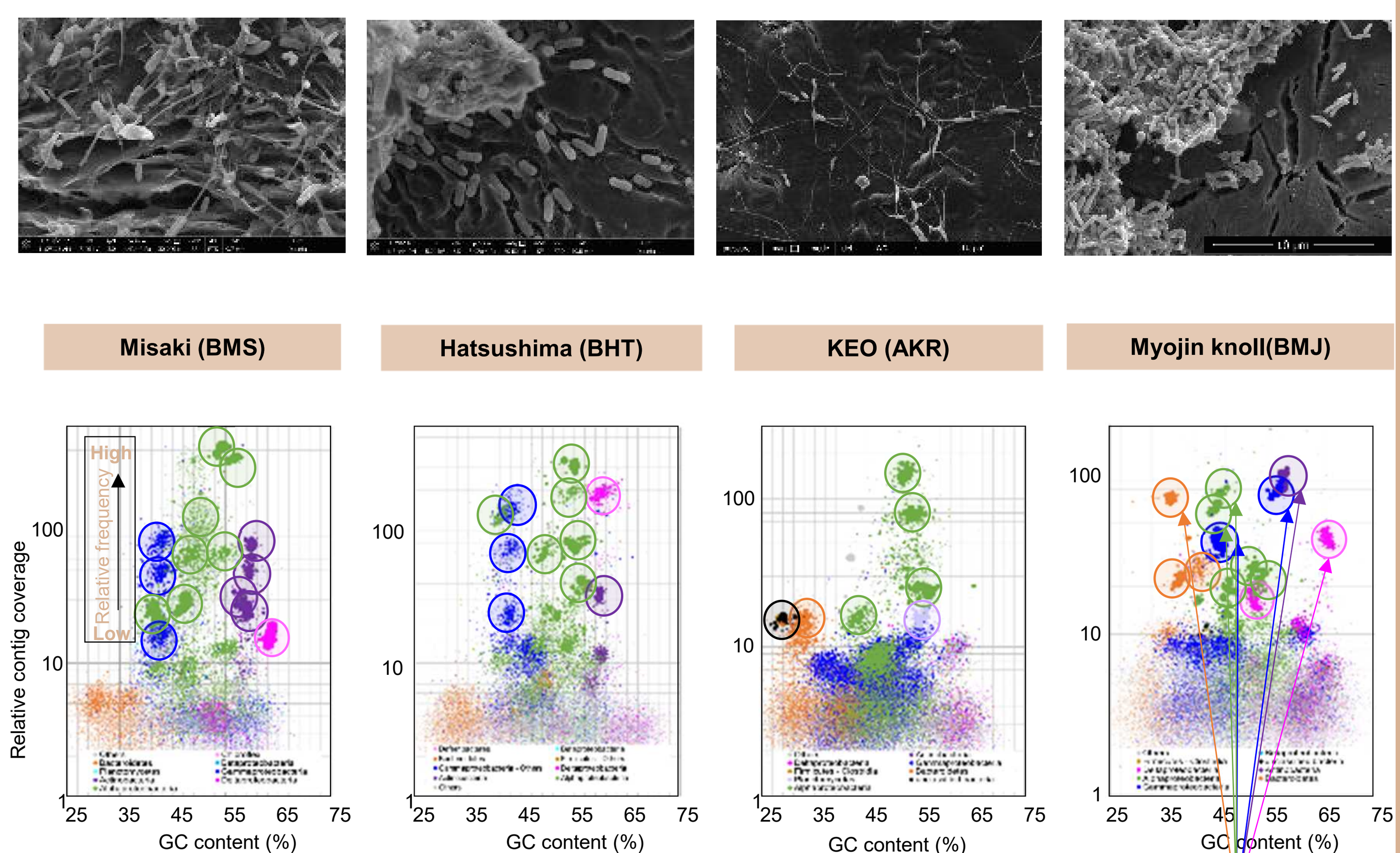
Meta-omics analysis of plastisphere correlating with biodegradation



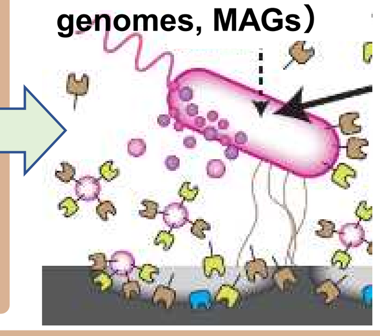
A method was developed to efficiently and rapidly analyze large amounts of data.



The candidate of PHBV-degrading Gammaproteobacteria and their PHBV depolymerase genes were identified through meta-omics analysis.



- Different and diverse plastisphere microbiomes formed on a marine biodegradable plastic at different sea area.
- Genomes (MAGs) of dominant microbes were recovered from the plastisphere metagenomes.
  - ➔ Growth of microbes related to biodegradation of plastic
  - ➔ Toward identification of enzymes for marine biodegradation



Genome of dominant microbes (Metagenome-assembled genomes, MAGs)

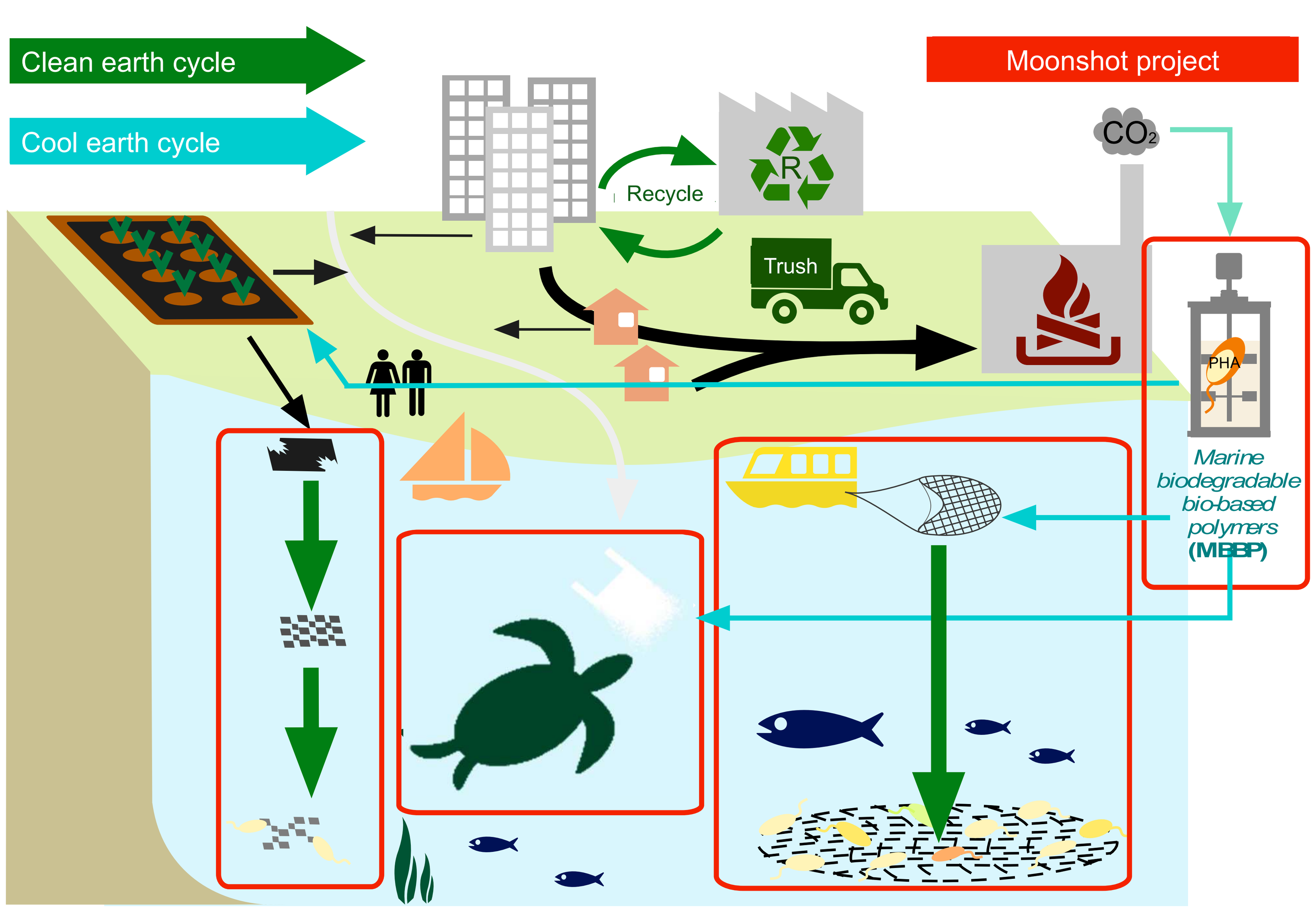
No: A-16-6E
PJ: R & D of marine biodegradable plastics with degradation initiation switch function
Theme: Research and development for social implementation (SI)

Organization: Gunma Univ, U Tokyo, Tokyo Tech, RIKEN, JAMSTEC
Contact: Gunma Univ Ken-ichi Kasuya(kkasuya@gunma-u.ac.jp)

NEDO
MOONSHOT

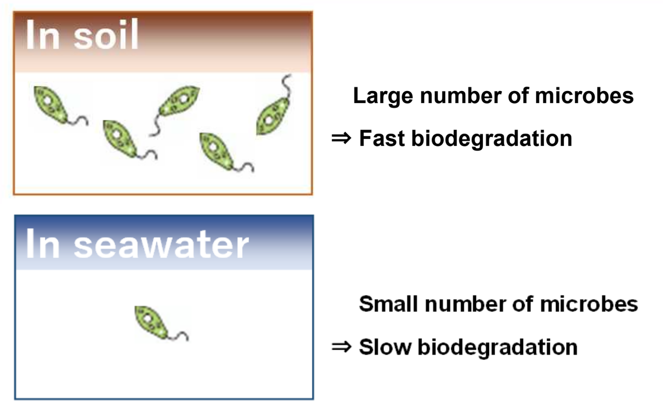
E1 E2 E3 E4 SI

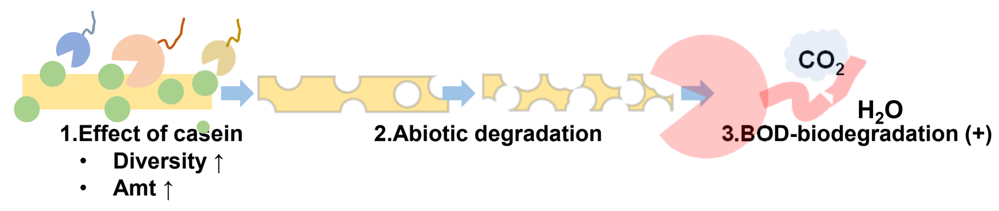
Social implementation of developed technologies



Controlling the biodegradation of plastics in the ocean by some compounds

NISSHINBO





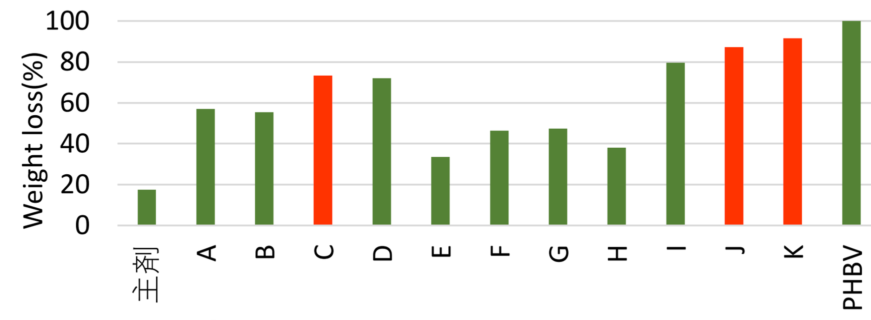
Search for marine degradation accelerators that attract microorganisms and accelerate degradation

Selection of L compounds

Effective utilization of food-waste

Candidates
：Soybean-derived mass-produced products,
Rice bran, ...

Collapsibility test of resin added with L compounds (A-K)(Water tank, 3 months, 10% of each additive)



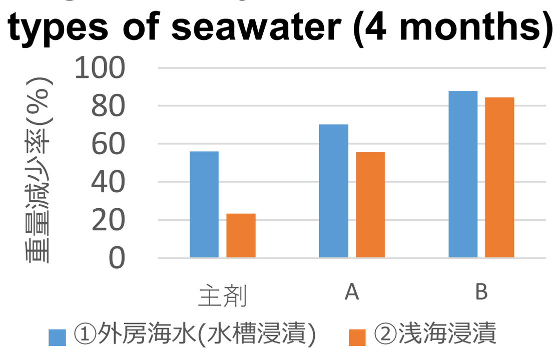
Establishment of technology for dispersion of degradation accelerator(L compound)s in biodegradable resins and molding technology

- Film molding by inflation method (assuming packaging films, etc.)
- Improvement of dispersion by optimization of composition and blending of the third component

⇨ Combination of decomposition and initial mechanical properties*.

＊： JIS Z1702

Degradability in various types of seawater (4 months)

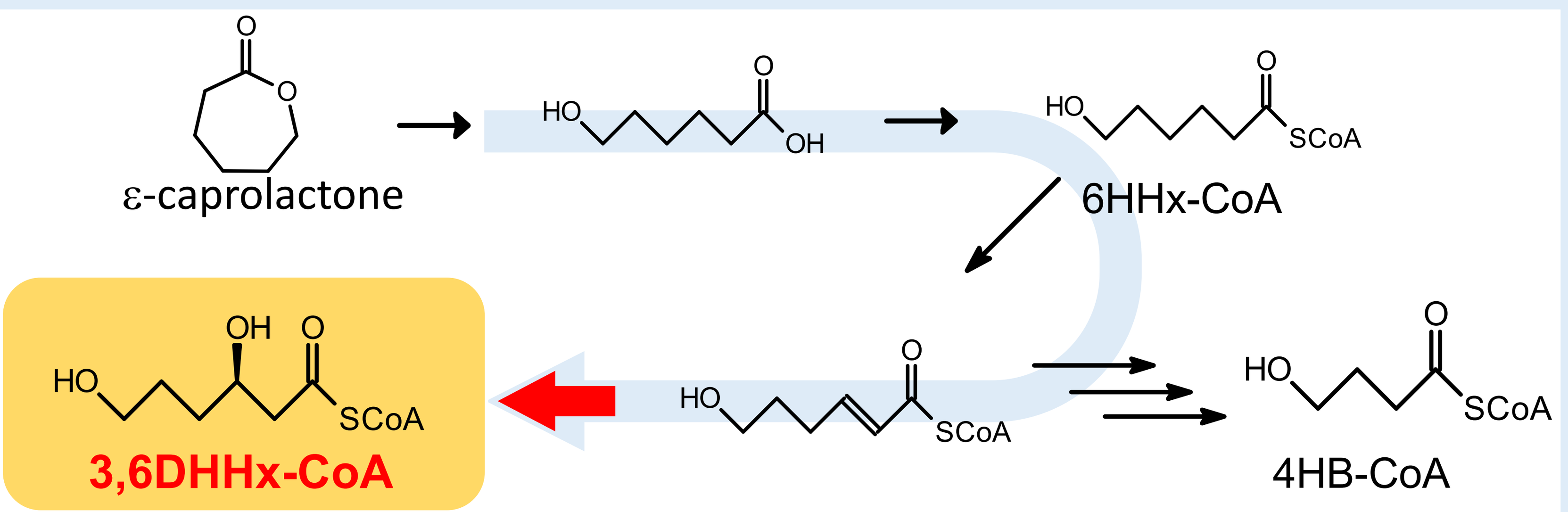


A:Soy-derived products 10% B: Soybean-derived product 10% + 3rd component 5 % (Sample thickness: 50 μm)

Synthesis of PHA for installing switching functions

＜Biosynthesis of PHA with hydroxyl groups on the side chain＞

Kaneka



Develop PHA polymerases that efficiently polymerizes 3,6DHHx-CoA produced from ε caprolactone by metabolism

Acquisition of PHA polymerase mutants with 2- to 3-fold increase in 3,6DHH ratio

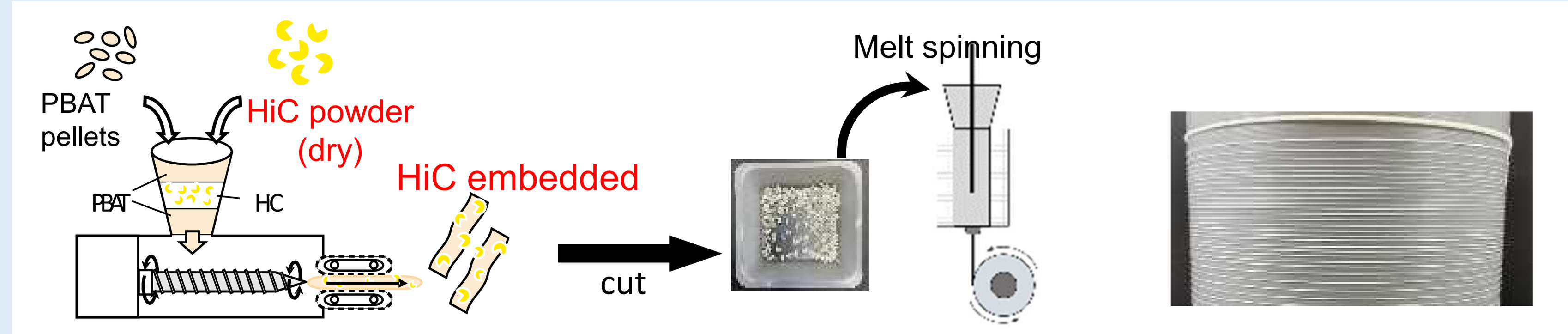
PHA synthases	Dry cell (g/L)	PHA (g/L)	Ratio of 3,6DHH (mol%)
Control	5.86	3.86	3.34
Mutant A	2.94	0.59	10.4
Mutant B	4.80	3.09	2.91
Mutant C	4.50	2.80	4.24
Mutant D	4.96	2.30	9.02
Mutant E	5.40	3.27	6.51

Host: *C. necator* mutant Carbon source: 15 g/L Fructose, 2.5 g/L ε-caprolactone

Development of manufacturing technology for marine-timed biodegradable fibers with properties that are practical for industrial applications

Marine degradability of high-strength fibers by utilizing the technology of embedding degradative enzymes in resins.

ニッケ

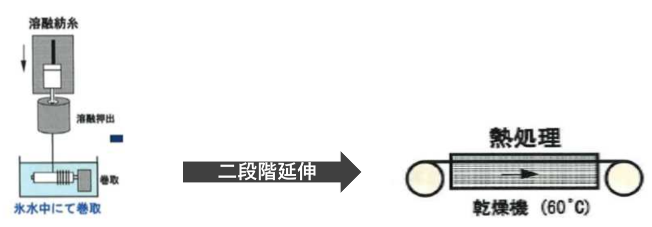


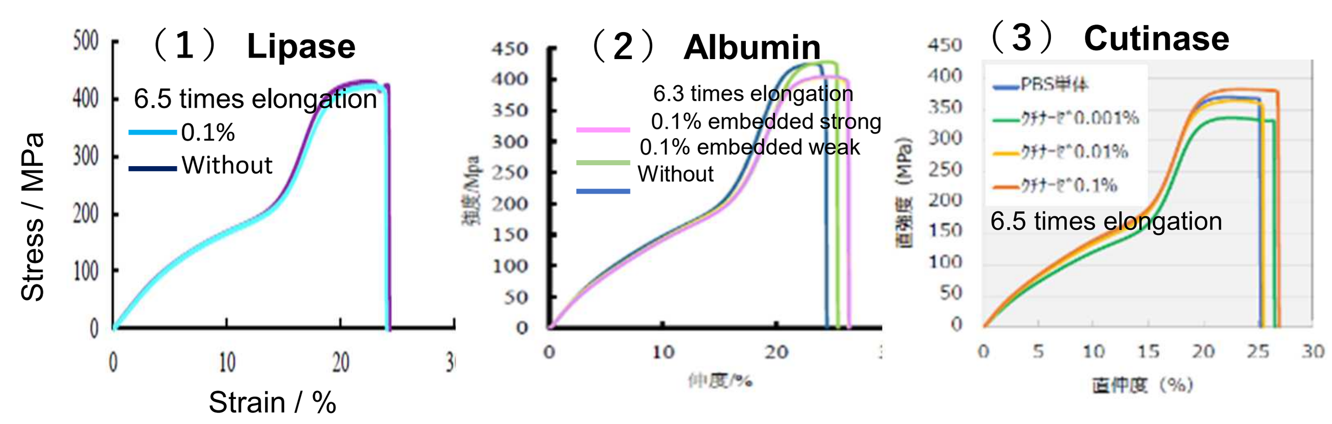
- **Biodegradable polyester fibers**

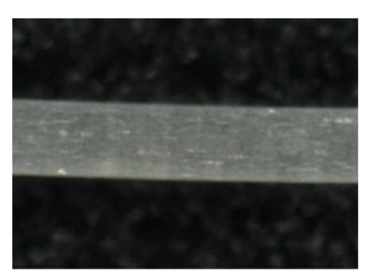
  PBS and PBAT were selected from the viewpoints of mass production and strength. 650 MPa tensile strength was achieved with PBS by optimizing the spinning conditions.
- **Development of enzyme production technology**

  The investigation of mass-producing enzymes with both thermostability and degrading activity is underway.
- **Development of enzyme addition technology**

  Optimization of enzymatic blending method has achieved suppression of decrease in fiber properties in enzyme- embedded (0.1%) PBS spinning.







PBS fiber with 0.1% cutinase

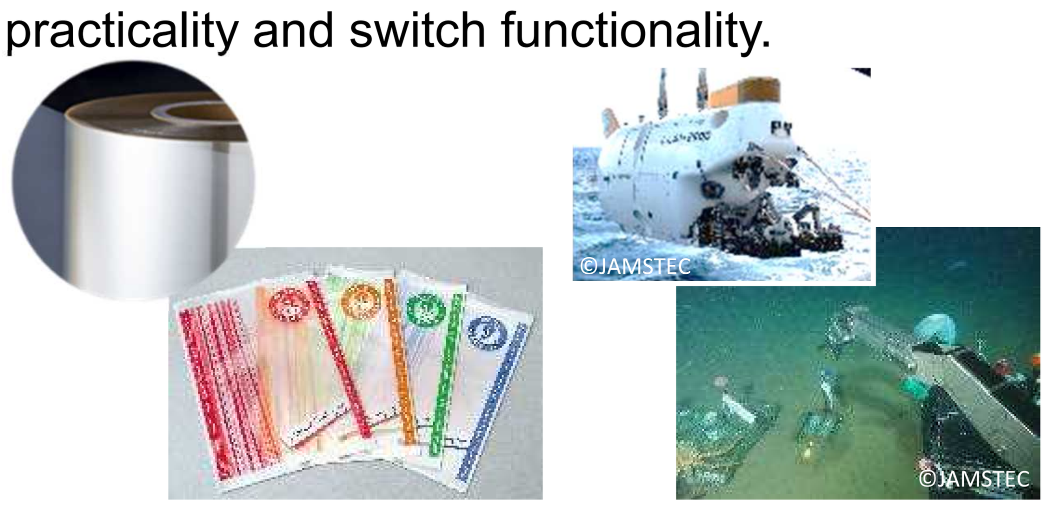
⇨ Marine biodegradability test will be conducted.

Collaboration with external partners

RENGO JAMSTEC 群馬大学 GUNMA UNIVERSITY

Evaluate the marine biodegradability of cellulose materials that have been given functions and composited as packaging materials

→ The aim is to achieve compatibility between practicality and switch functionality.



日本カーリット株式会社 Japan Carlit Co.,Ltd. 群馬大学 GUNMA UNIVERSITY

Development of evaluation methods for biodegradability of marine biodegradable plastics

Development of an analytical system capable of accurately measuring biodegradability employing a flow-type system and search for new materials with marine biodegradability.

Other cooperating companies for joint development, sample provision, etc.

DAICEL etc.・・・

Promote social implementation of marine biodegradable plastics based on the technology developed in PJ in collaboration with various companies.
To create a clean earth for the future