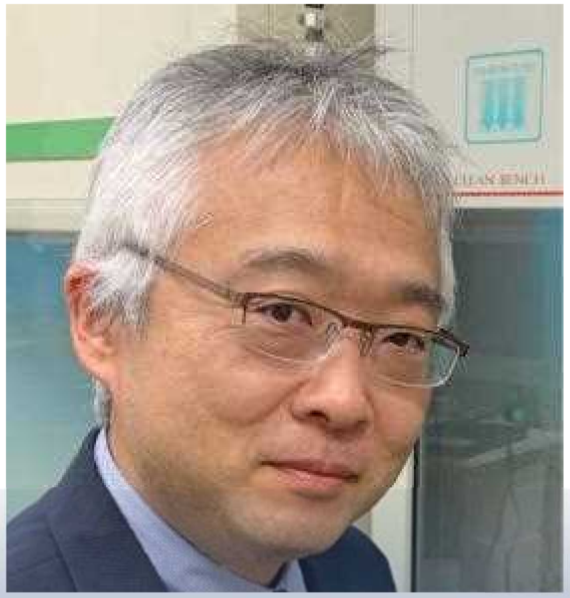
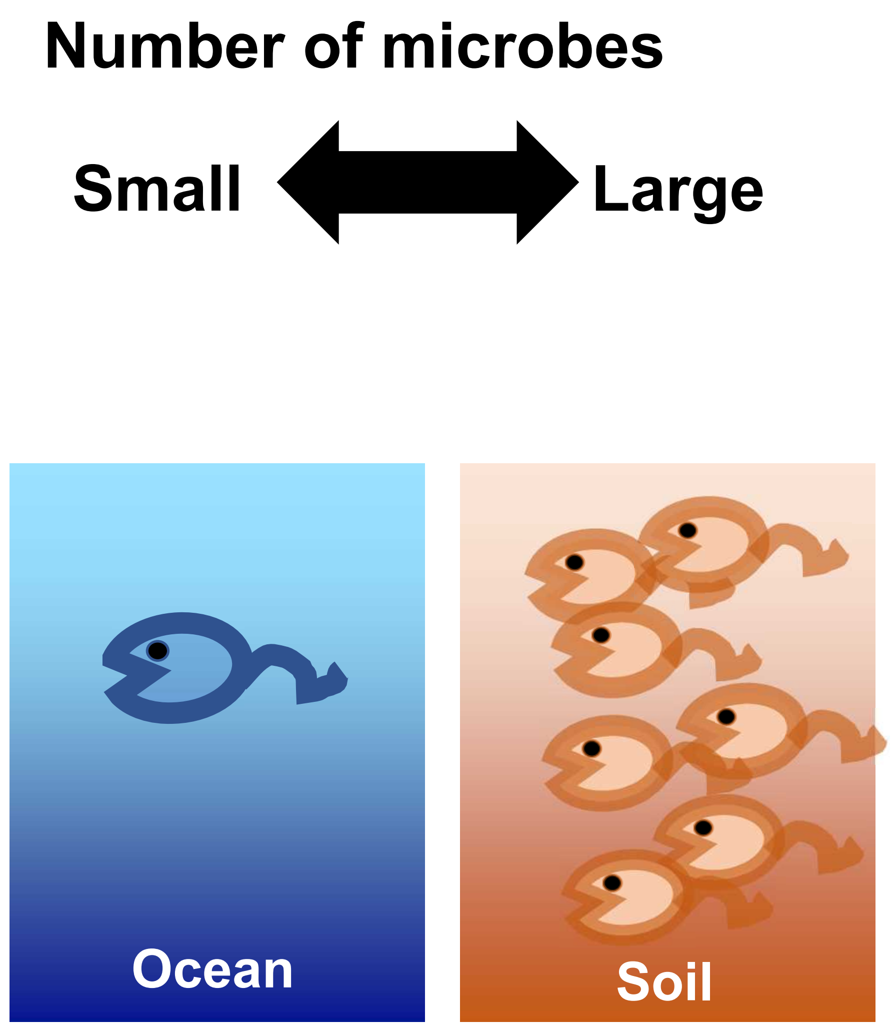
R & D of marine biodegradable plastics with degradation initiation switch function



PM：Ken-ichi Kasuya
Gunma Univ. Prof.
Organization: Gunma Univ, U Tokyo, Tokyo Tech, RIKEN, JAMSTEC

a. Background—Two challenges in marine biodegradable plastics



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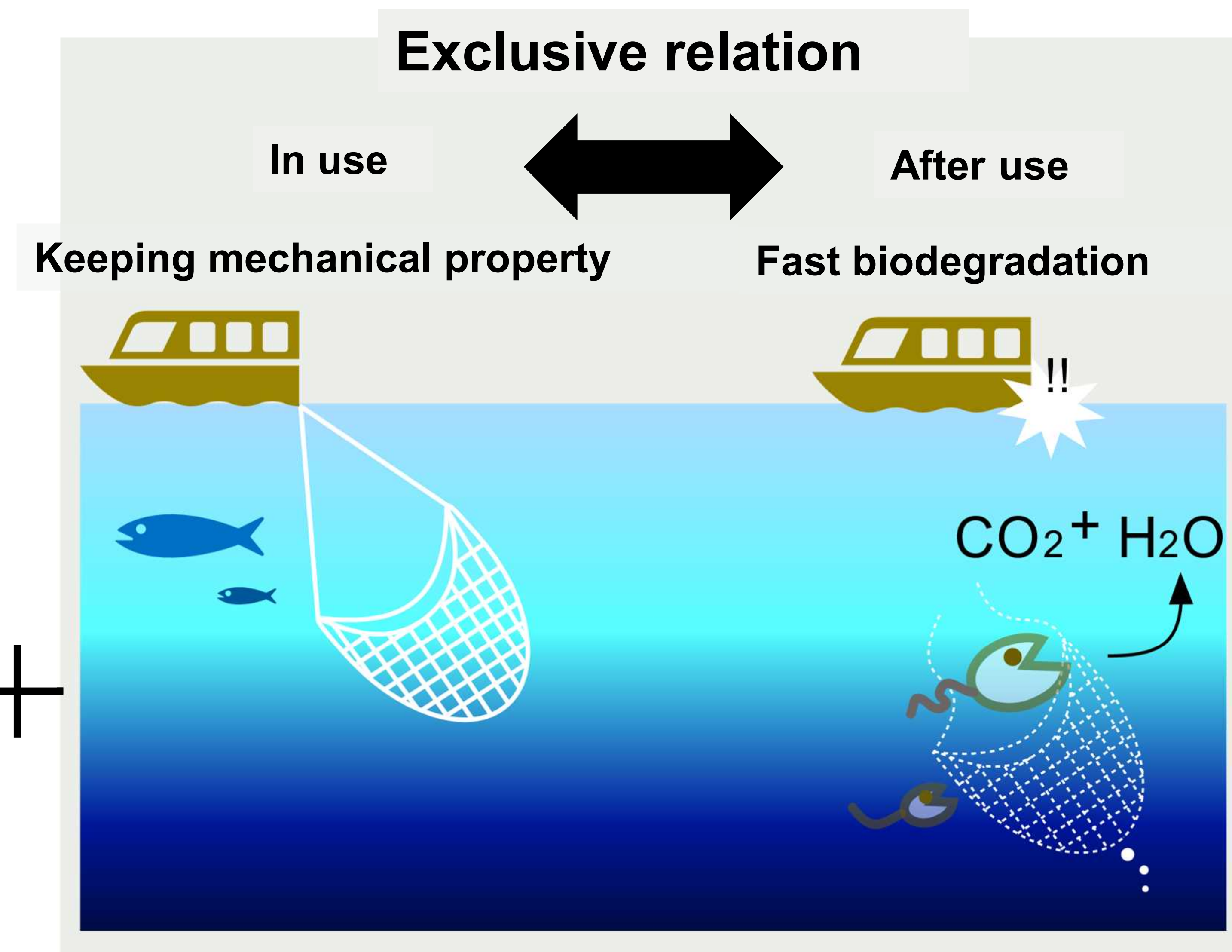
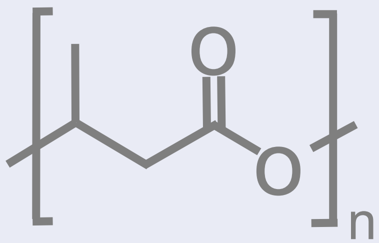
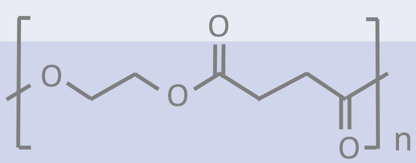
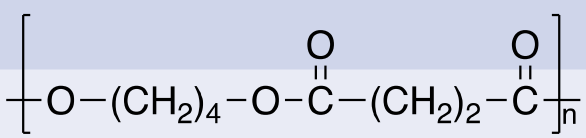
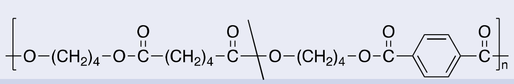
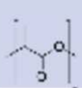
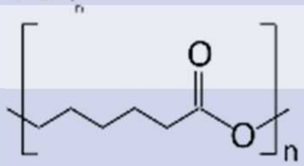


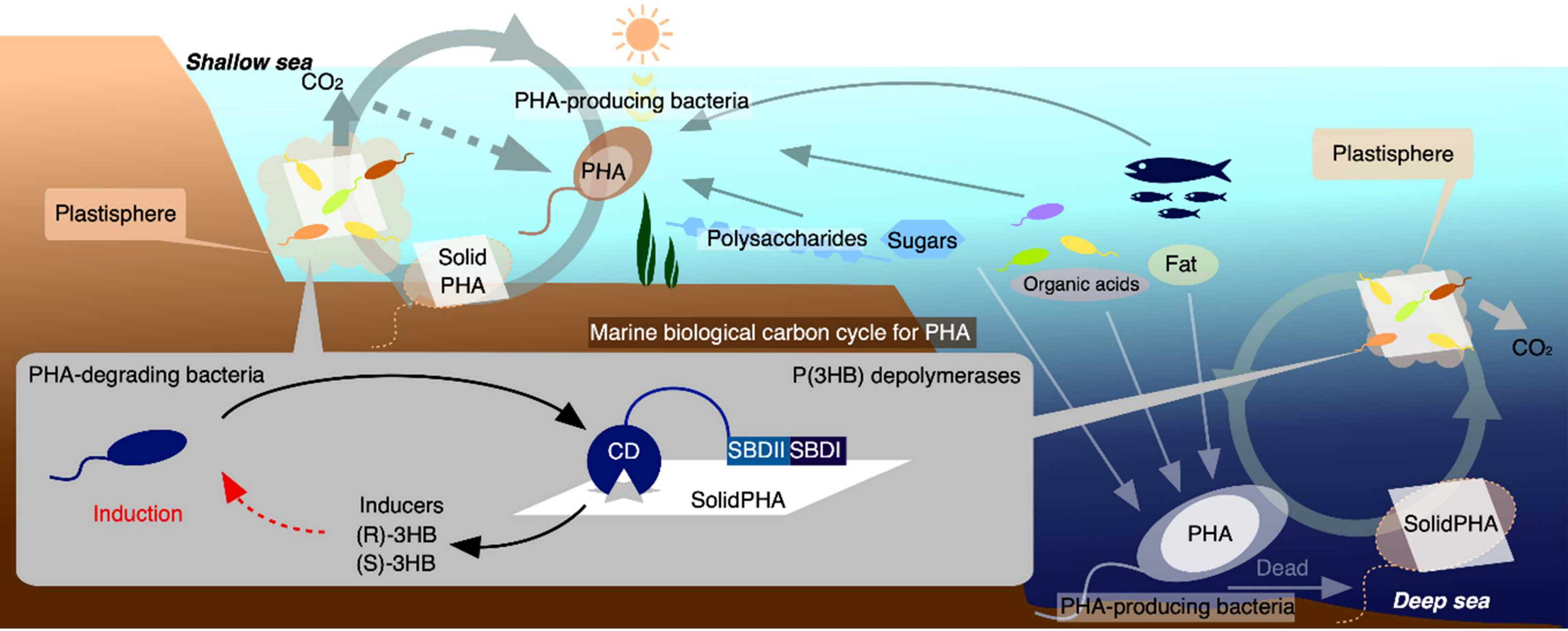
- The number of microbes in marine environment is small.
- Biodegradation of biodegradable plastics is very slow in marine environments.

- Biodegradable plastics are gradually biodegraded during use.

a. Background—Few marine biodegradable plastics

Polymers	Environmental degradability		
	Excellent	**Depending on site**	**Poor**
PHAs  Marine OK	Soil Freshwater Brackish water **Seawater** Aerobic sludge Anaerobic sludge Compost	-	-
PESu 	Soil Freshwater Compost Activated sludge	-	Seawater
PBSu  	Compost	Soil	Seawater Activated sludge Freshwater
PBAT 	Compost	Soil	Freshwater Seawater
PLA	Compost	Soil	Seawater
PCL  Marine OK	Soil Freshwater **Seawater** Compost	-	-

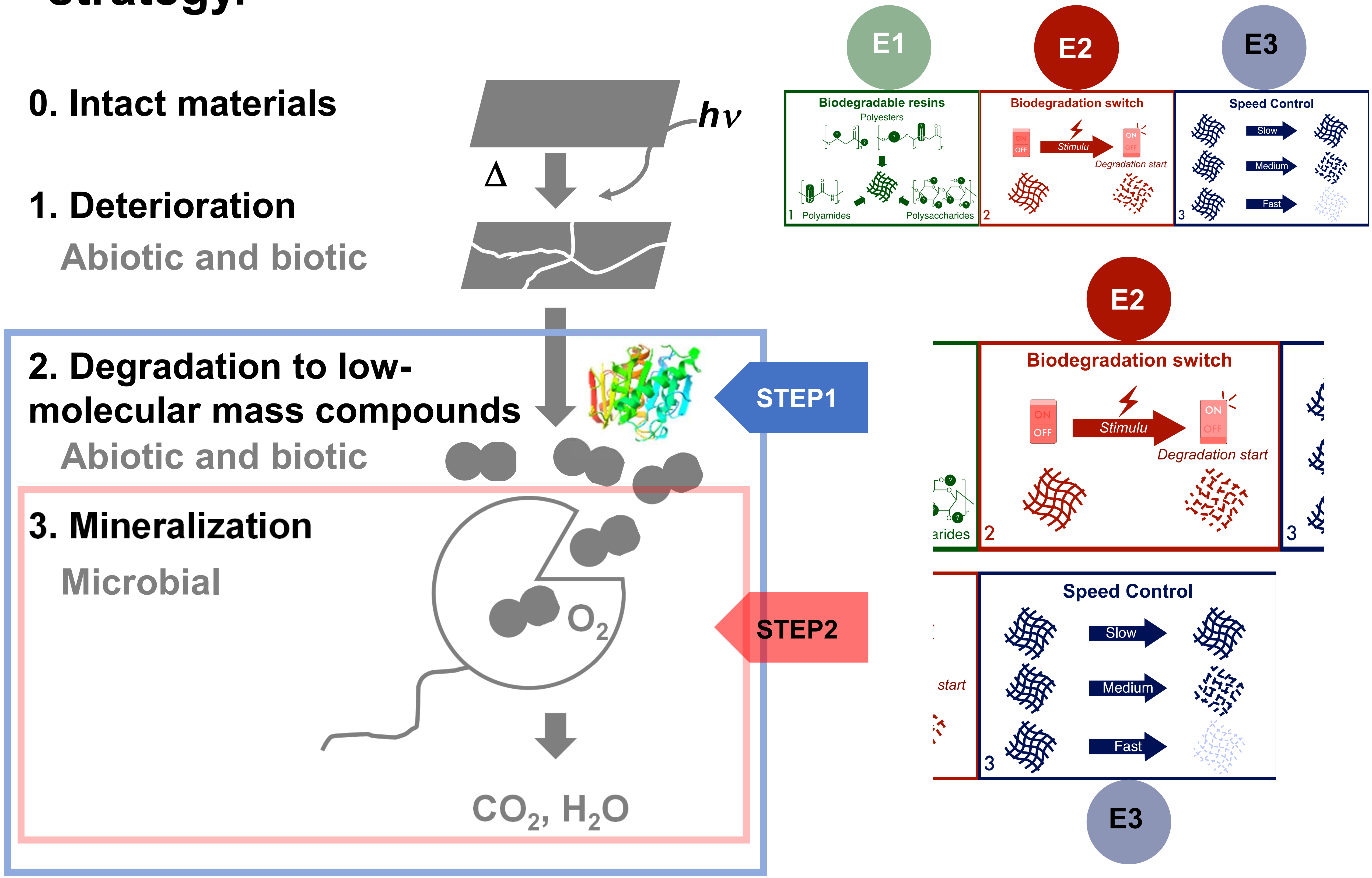
a. Background—PHAs are biodegraded in marine environments.



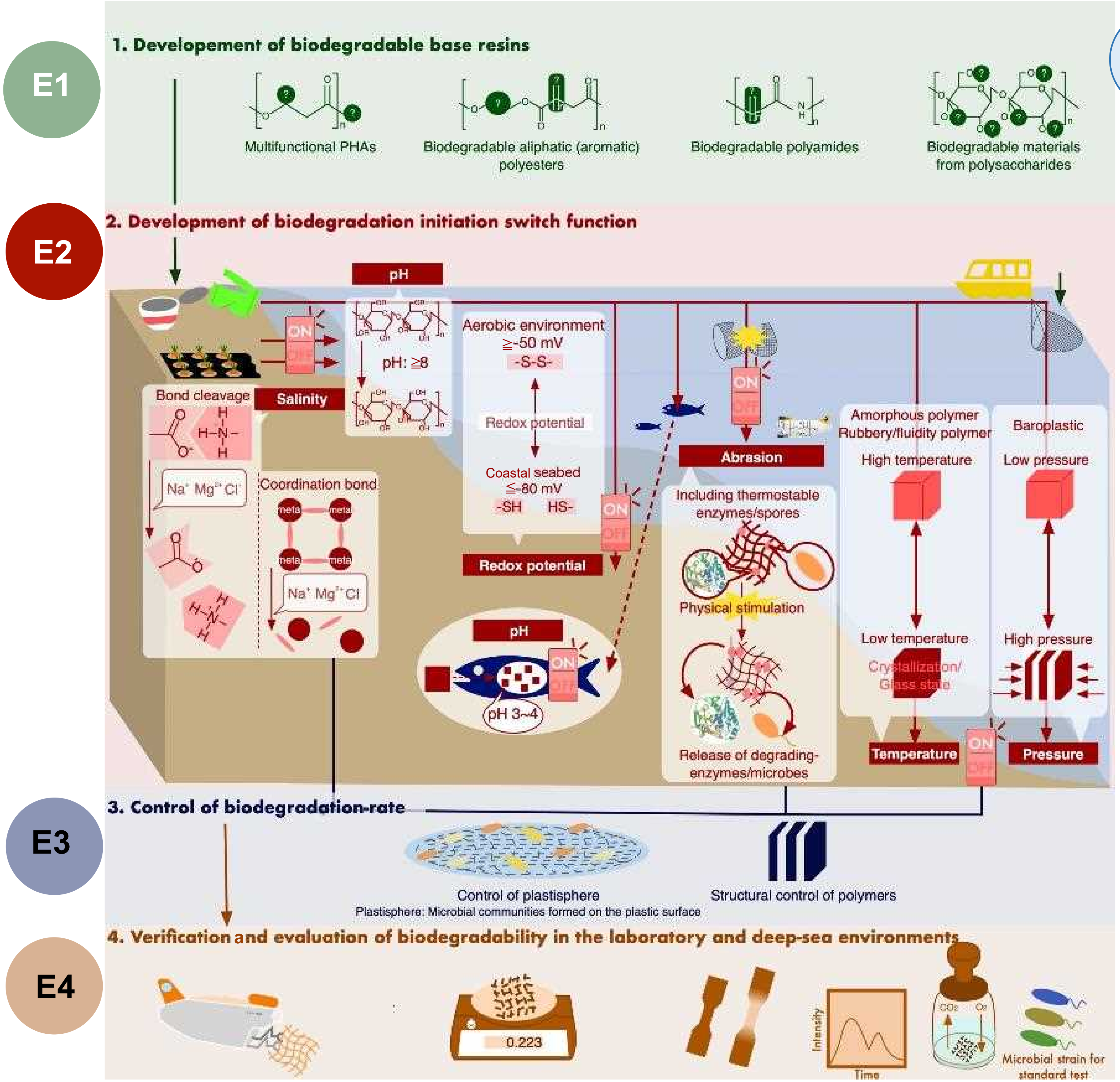
Polym J **53, 47–66 (2021)**

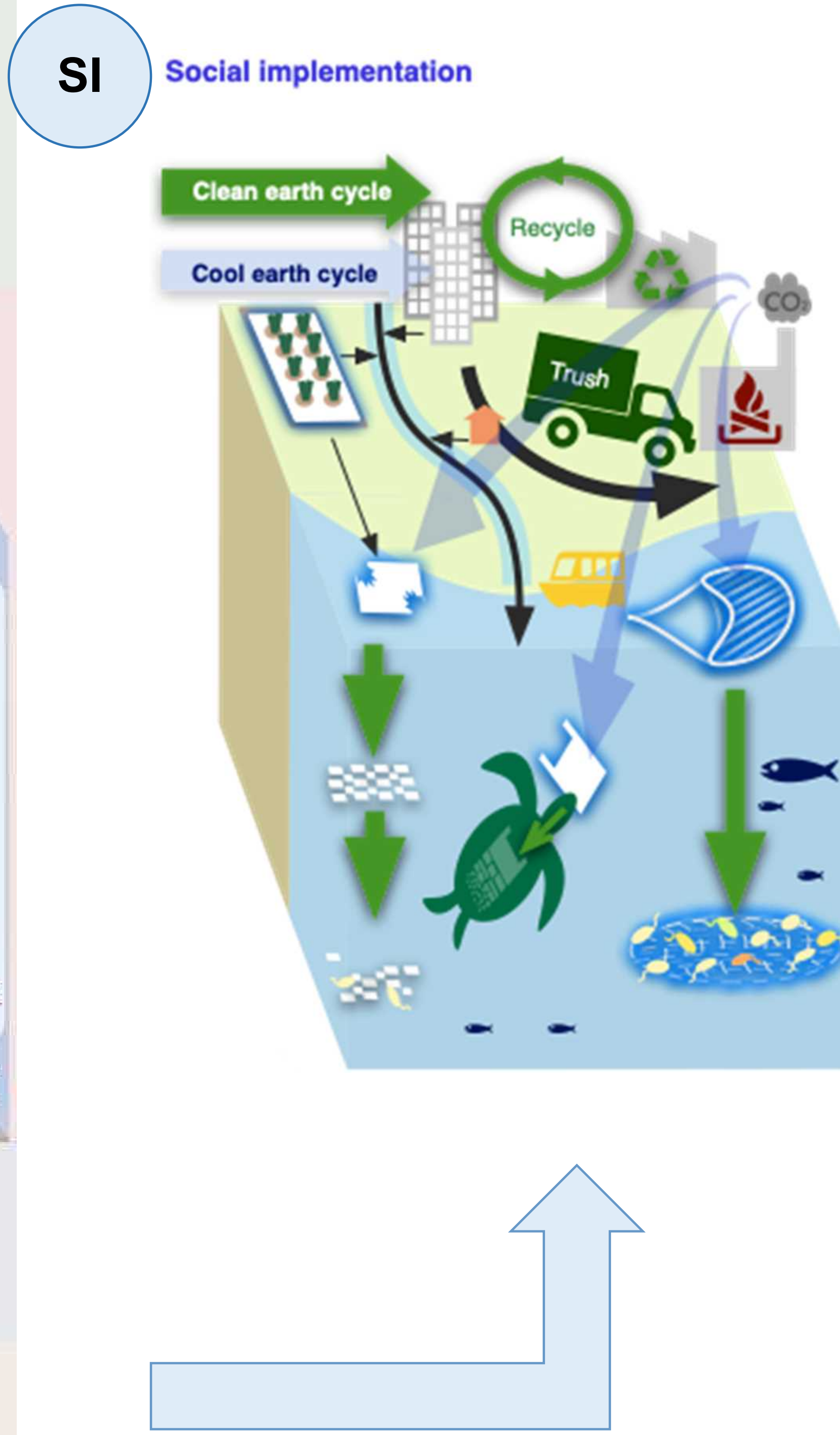
PHAs are produced and biodegraded in marine environments.
Biological carbon cycles for PHA exist in the marine environment.

a. Background—Biodegradation mechanism and R & D strategy.

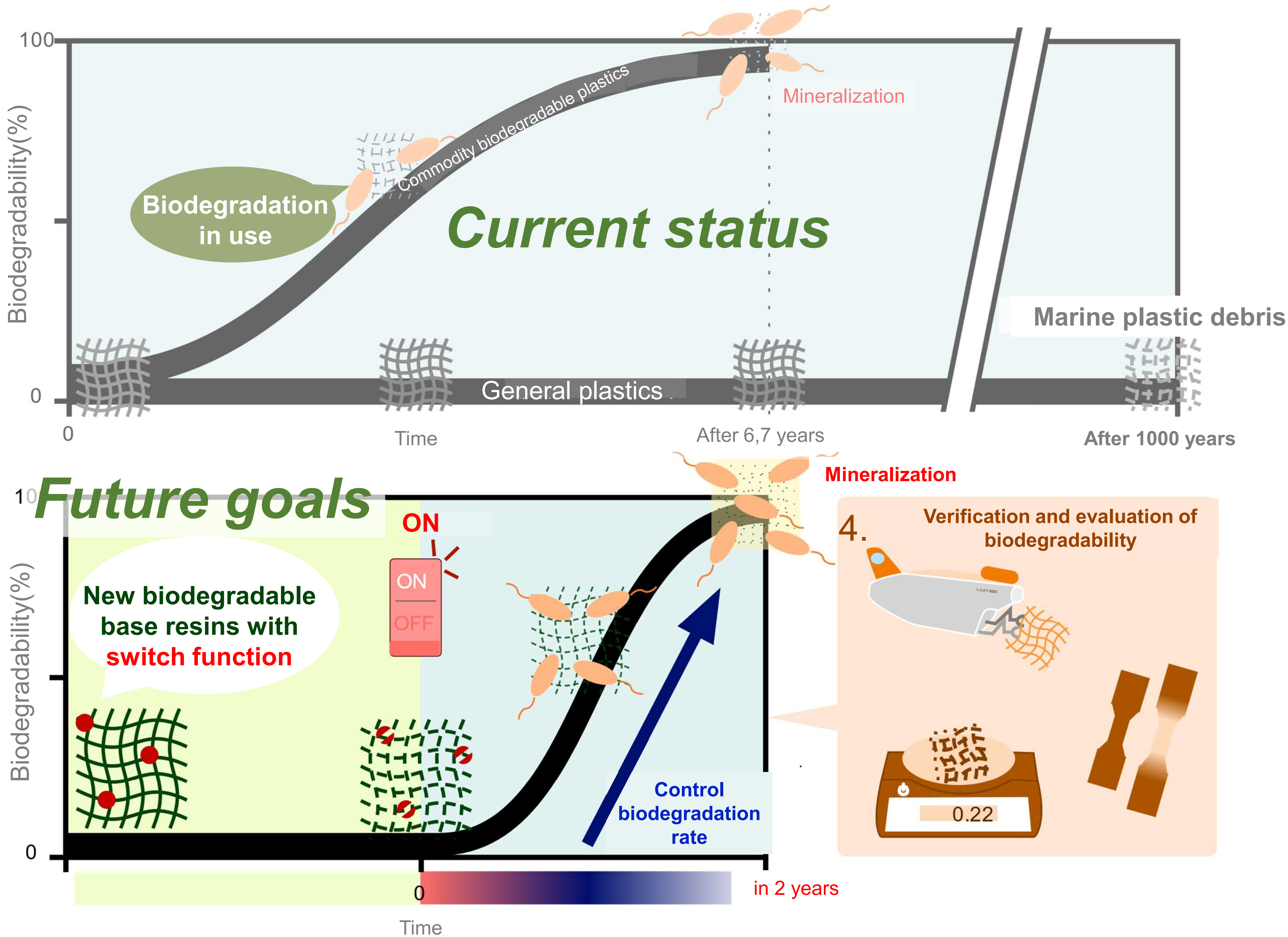


1. R&D items of the project（E1, E2, E3, E4, SI）

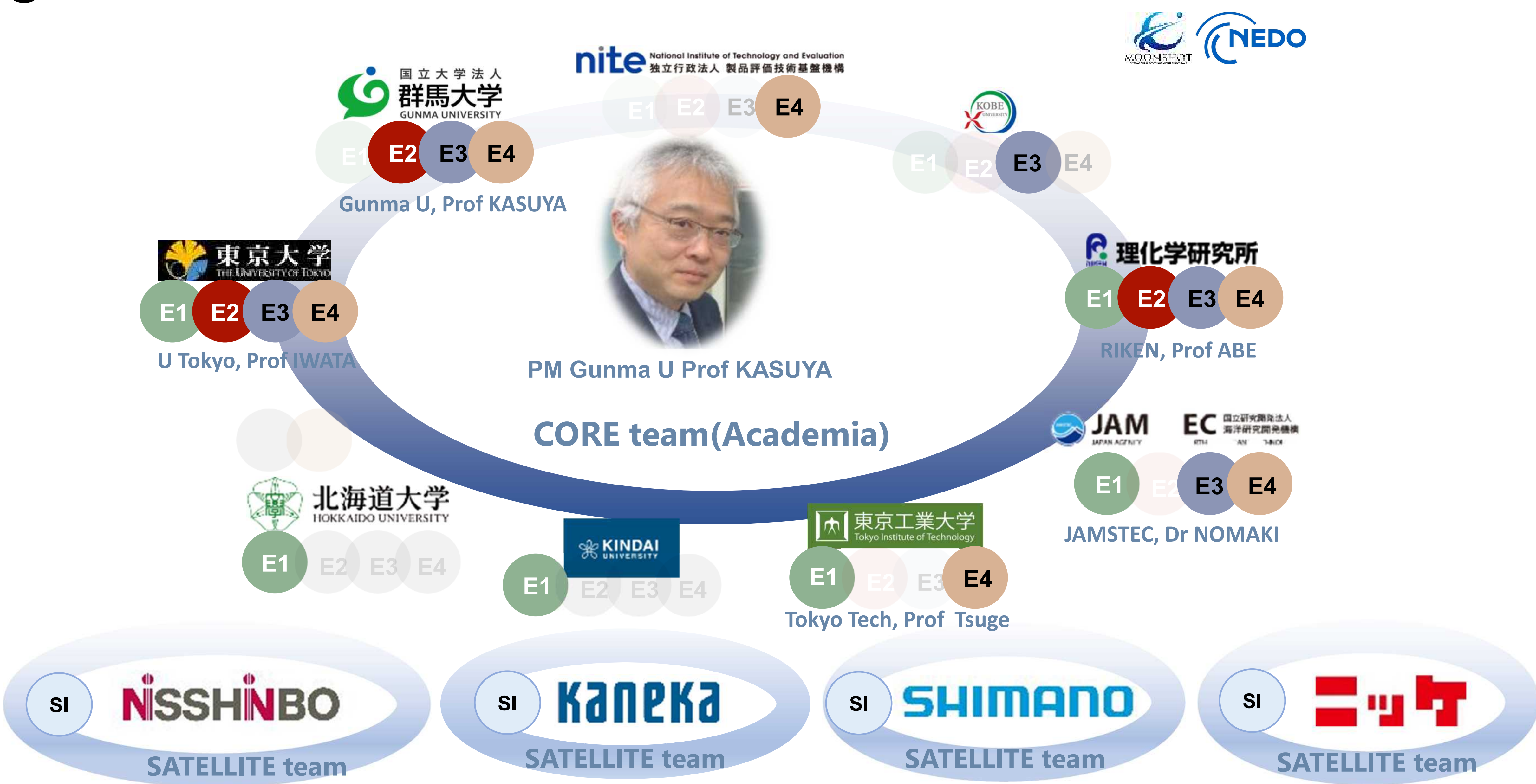




2. Current status and future goals



3. Organization of R & D



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 outcomes.

Other external cooperating companies

GPI ゼネラル・パッケージング・インダストリー レンゴー

日本カーリット株式会社 Japan Carlit Co., Ltd.

DAICEL

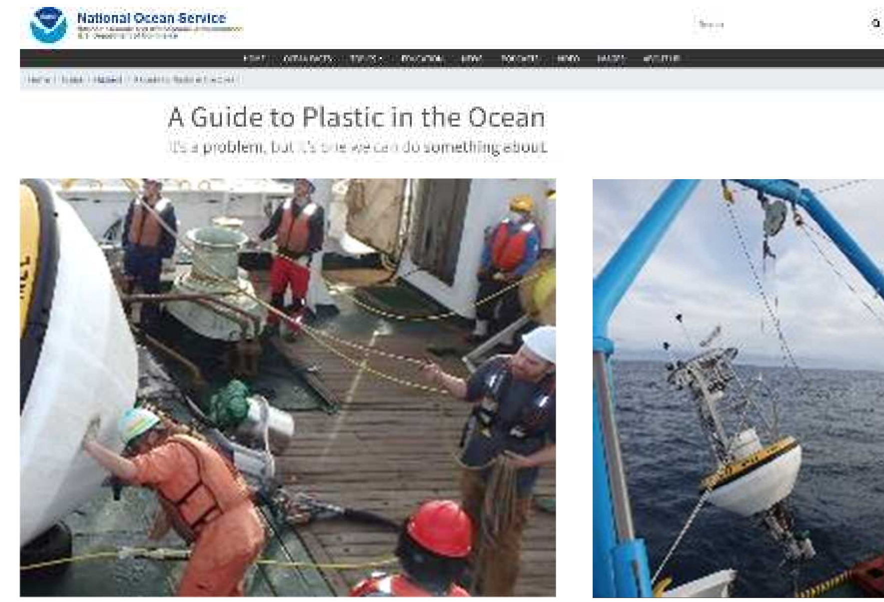
4. International cooperation and science and technology dialogue with the public

Research Team ★

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

Research Subject

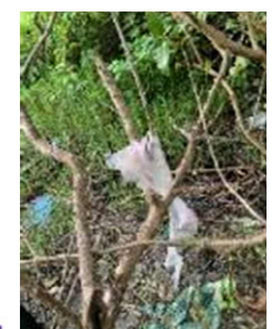
Biodegradable plastic ocean surface mooring experiment on the NOAA observatory buoy



Installation of developed materials on NOAA buoys

Research Team 2 : ★

Sudesh Kumar, Professor,
Universiti Sains Malaysia (USM)



Plastic debris in mangroves

Evaluation of biodegradation of materials developed in the PJ in mangroves

Research Subject

Evaluation of biodegradation of biodegradable plastics on tropical mangrove forests.

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)**

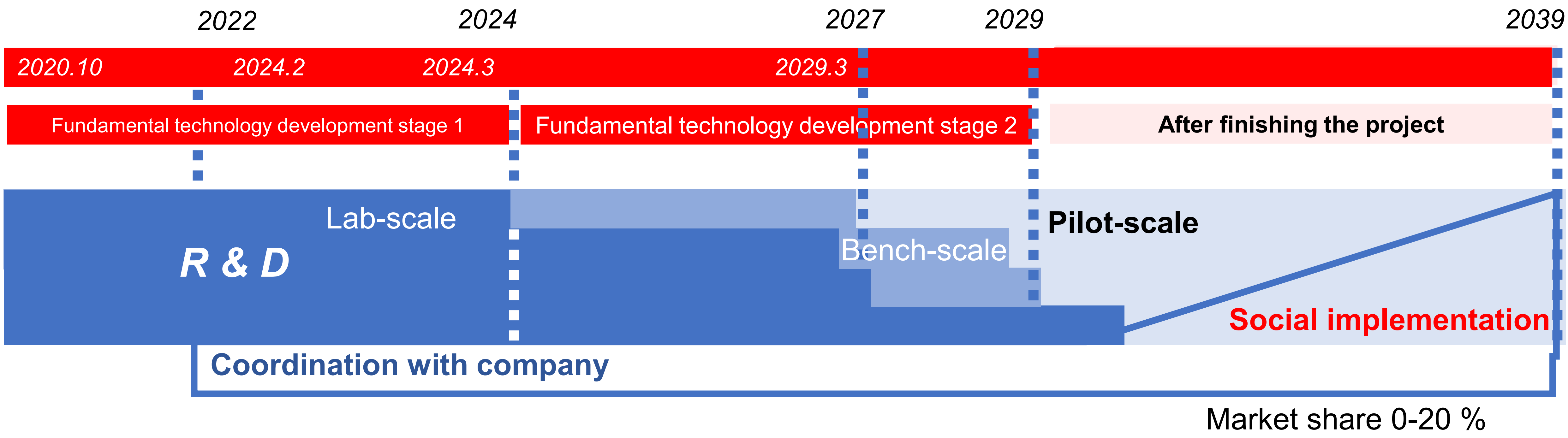
4. Science and technology dialogue with the public



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.

5. Time schedule for R & D

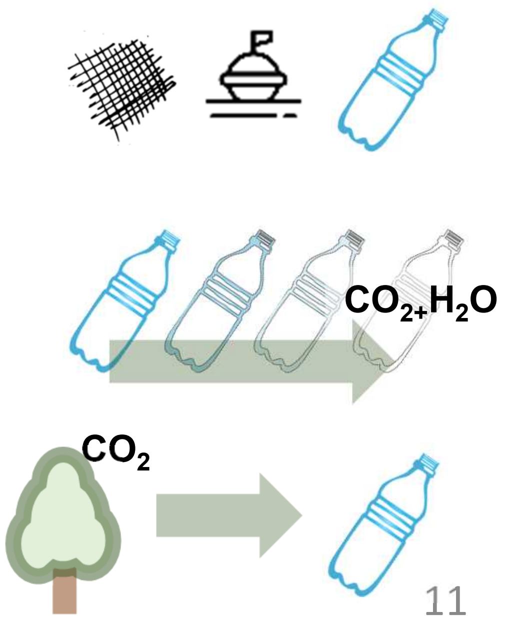


6. Goal of our project（2029）

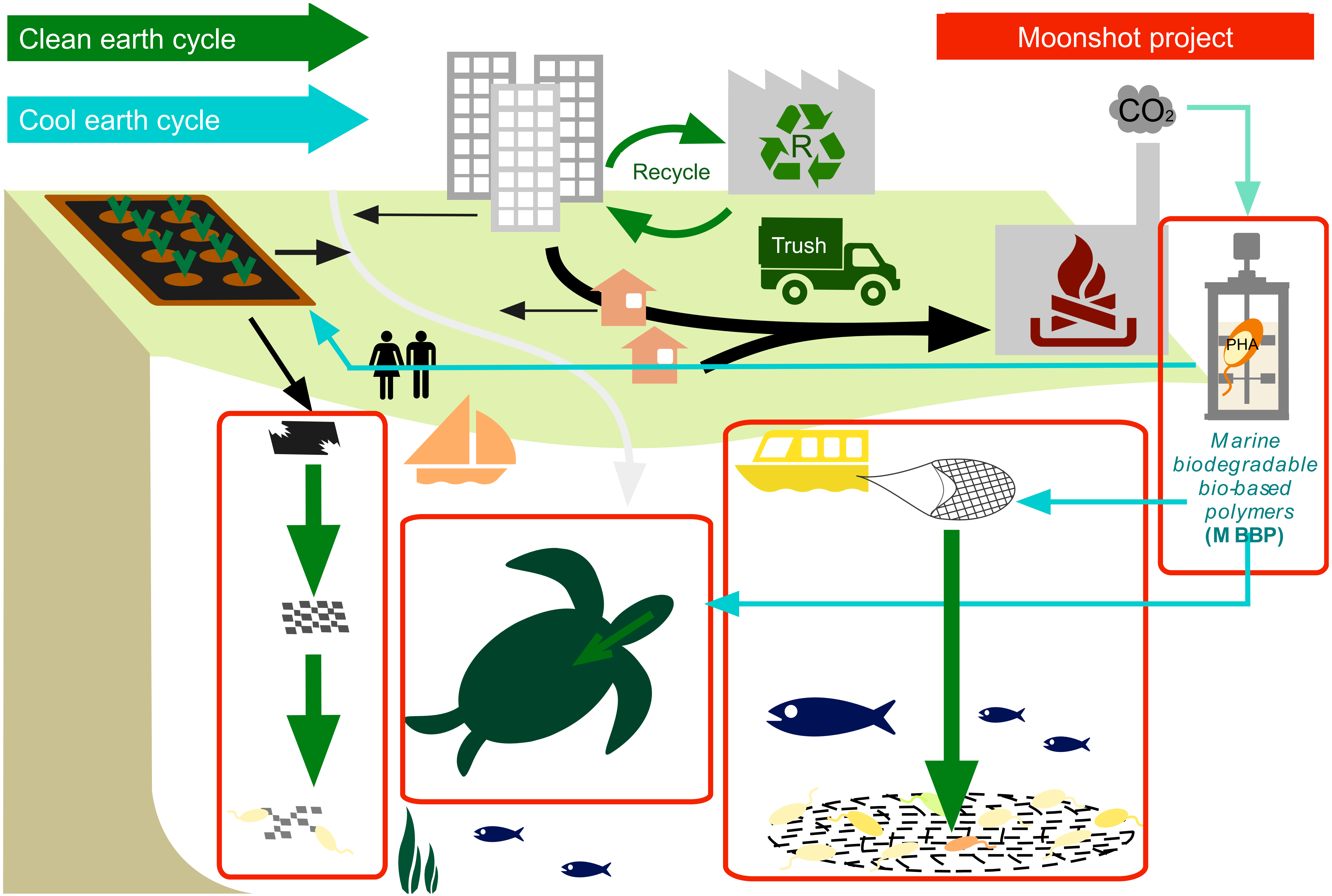
① We create three or more new marine biodegradable plastics that exhibit 90% biodegradability in seawater at 30 ˚C in 6 months after the switching function exerts.

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.



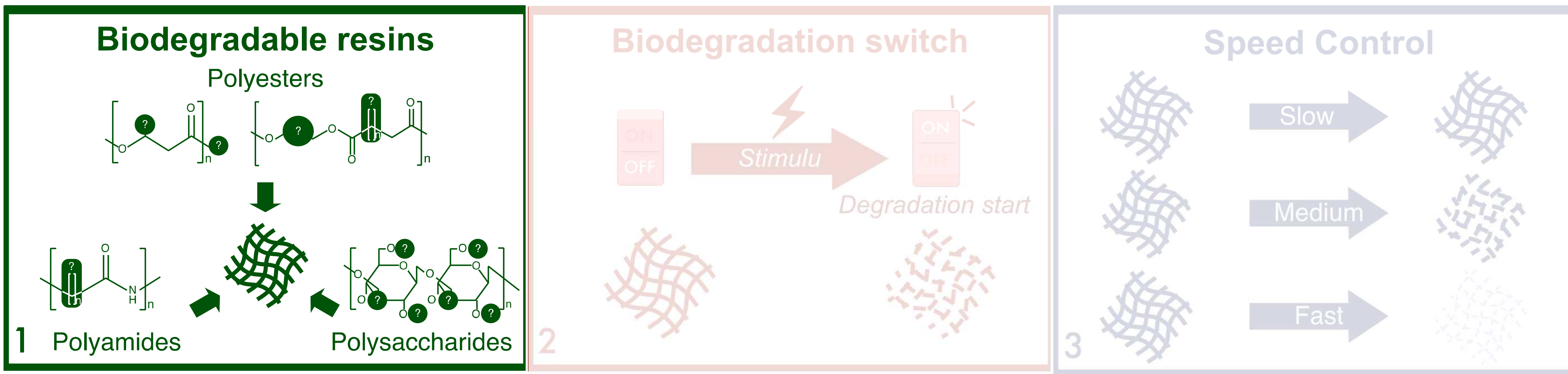
7. Social implementation



E1

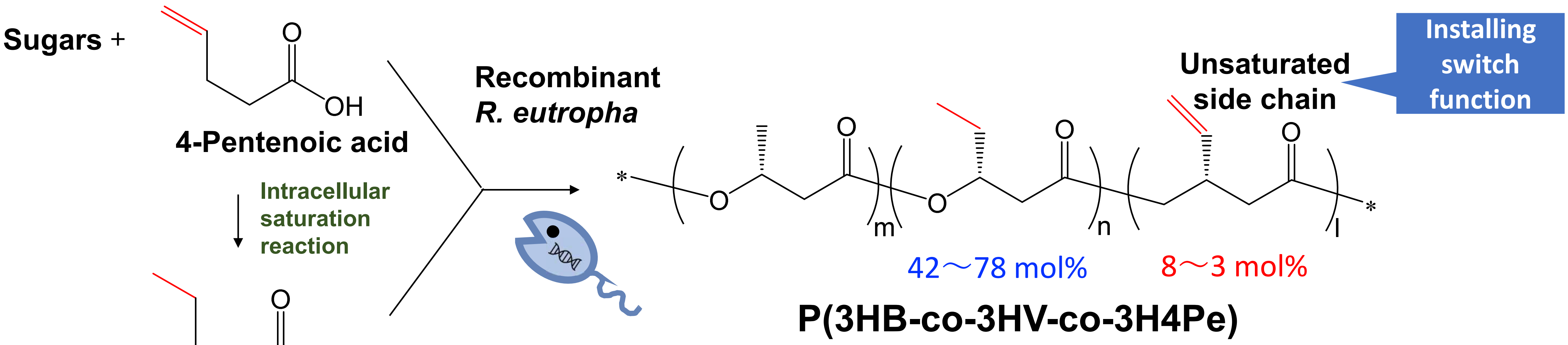
8. Major results regarding ongoing topics

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



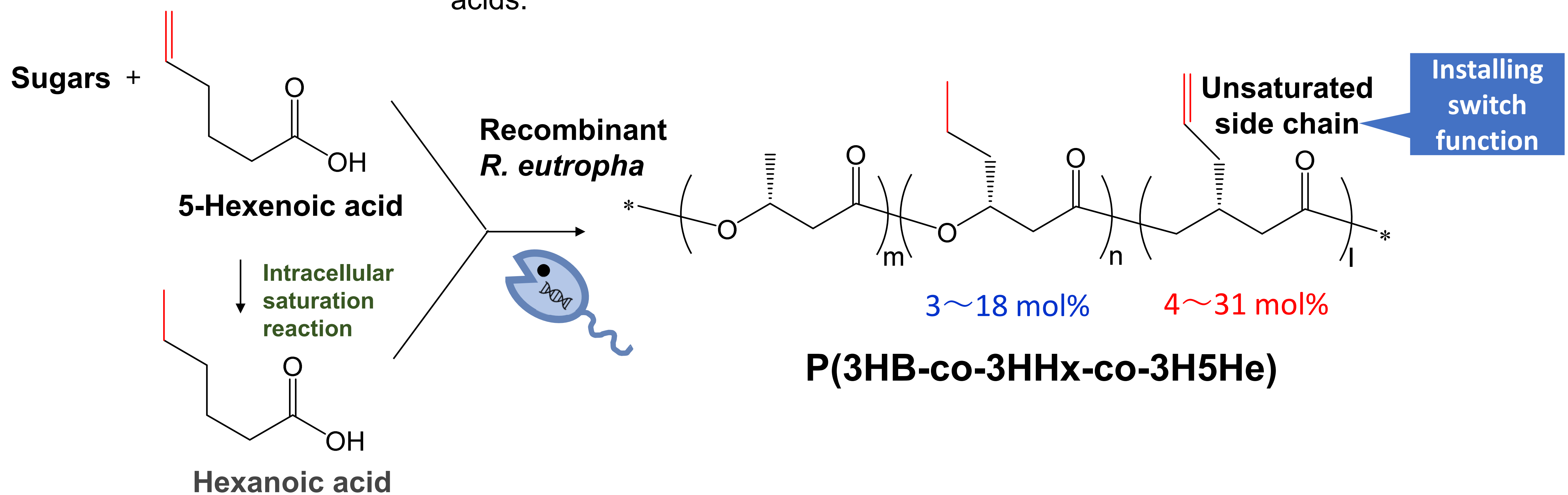
E1：	R & D of marine biodegradable base resins	**PHA**	Functionalization Toughening Fixation of carbon dioxide
		Polysaccharide	Plasticization/Biodegradability Toughening
		Synthetic polymer	Biodegradable building-block polymer

E1 Base materials for installing switching functions

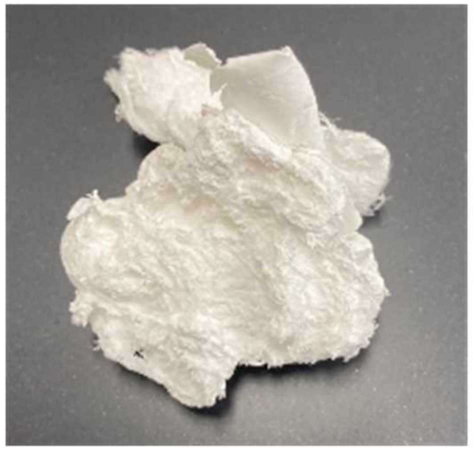


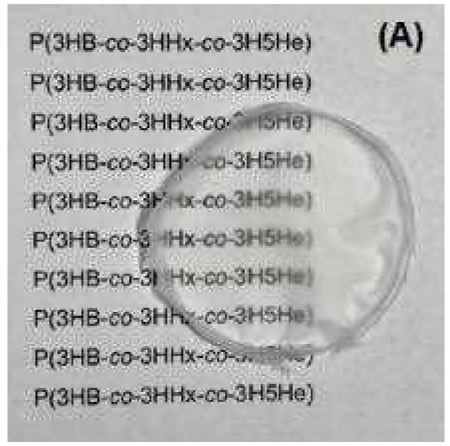
Hexanoic acid

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



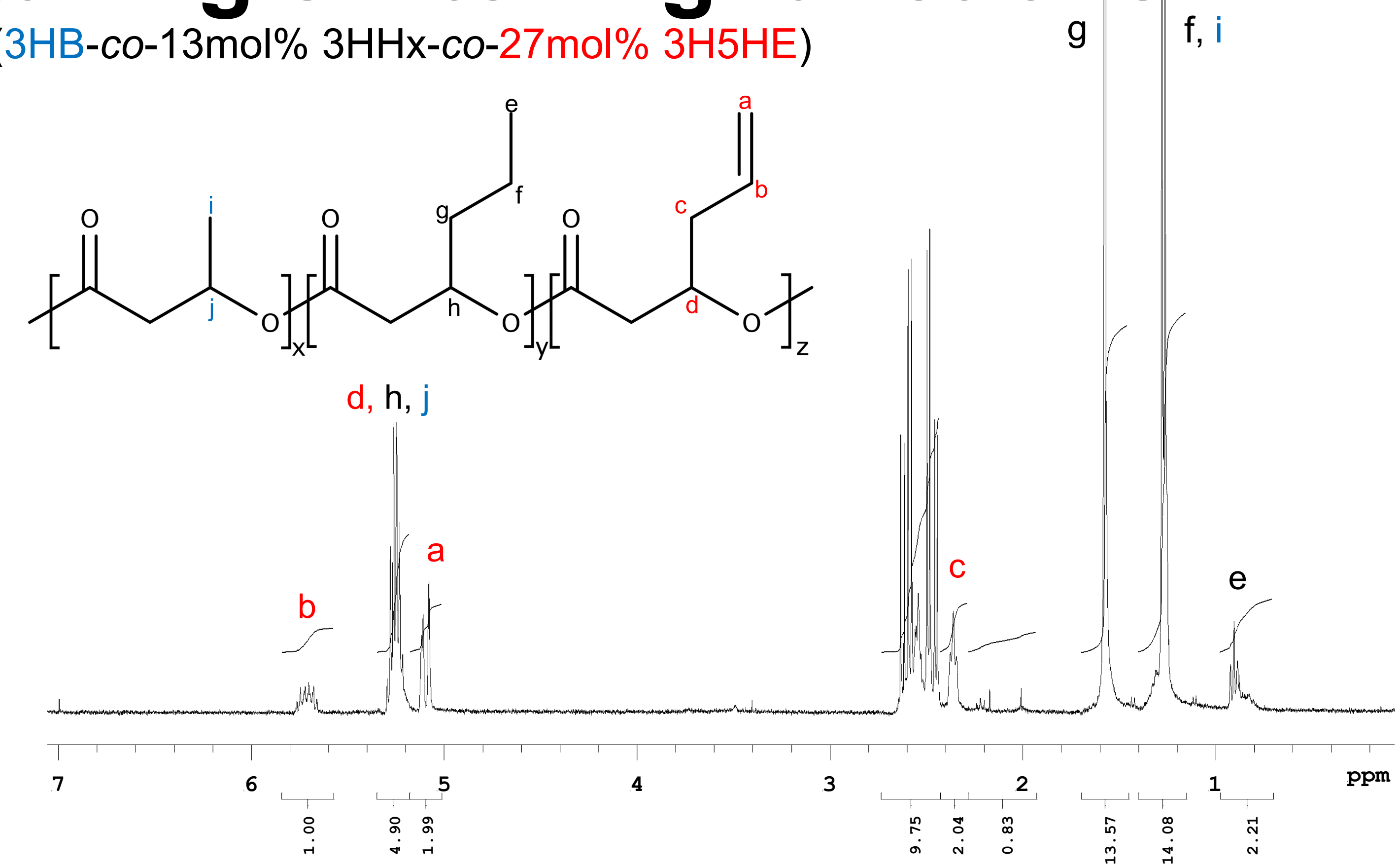
E1 Base materials for installing switching functions

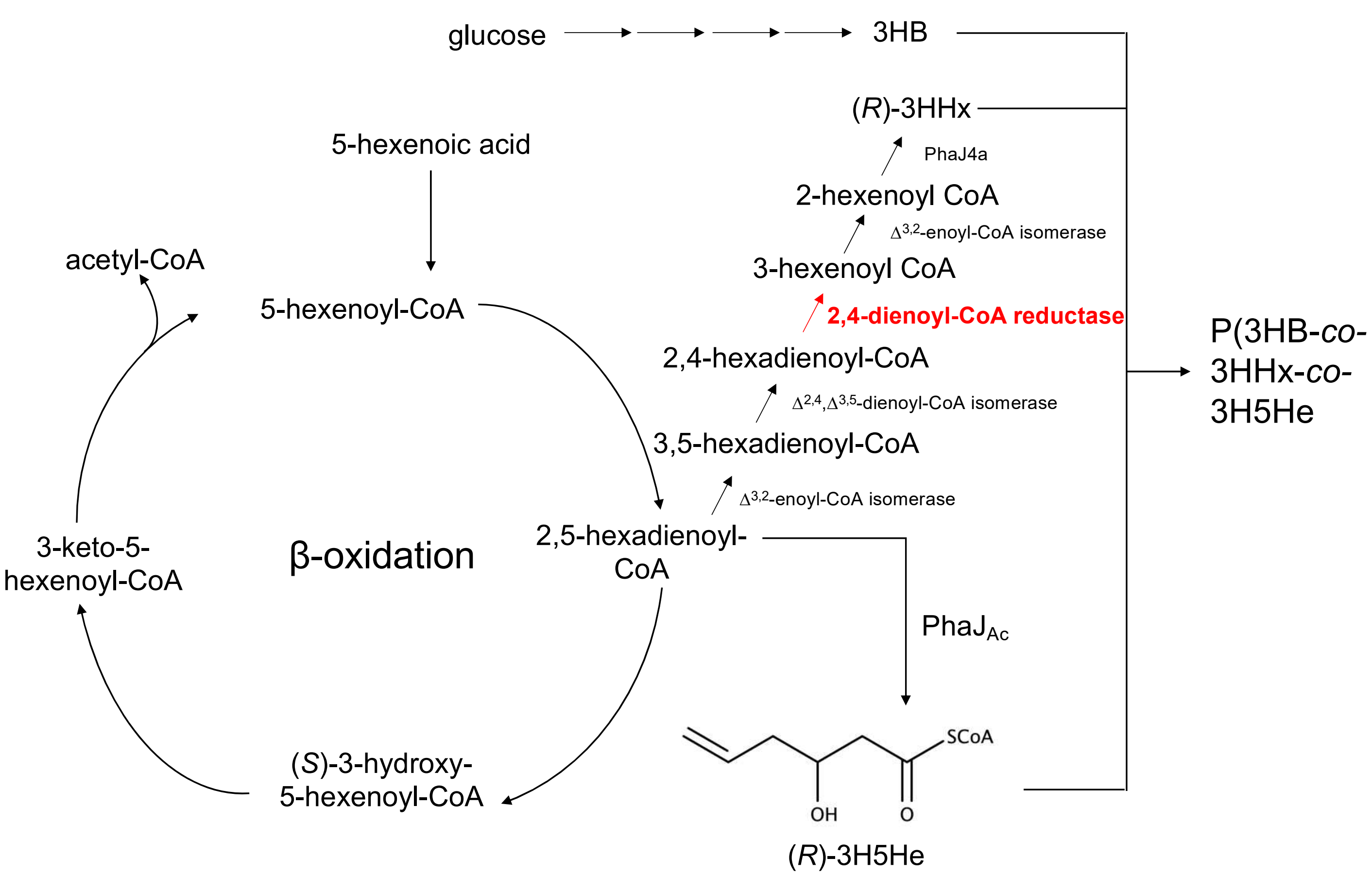




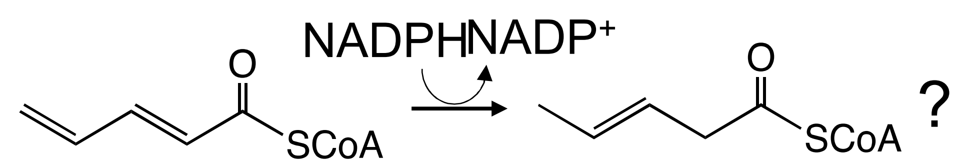
P(3HB-*co*-15.8% 3HHx-*co*-9.0 mol% 3H5He)
Mw: 28.9 x10^5, PDI: 2.4, Tg: -1.6℃, Tm: 119℃
Tensile strength: 14 MPa,
Elongation to break: 1,158%

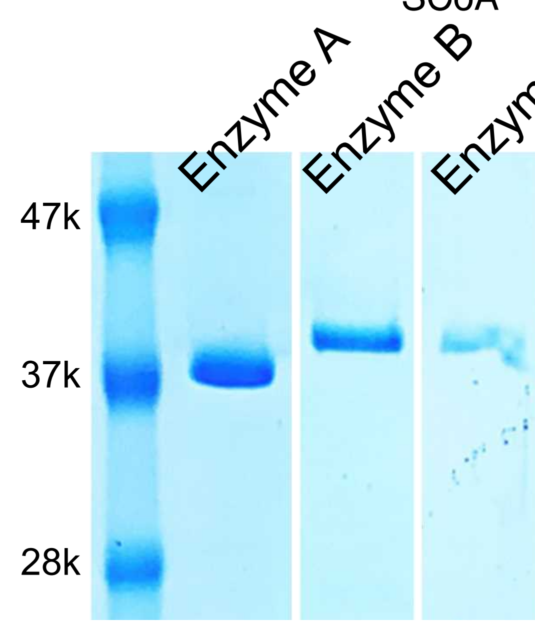
P(3HB-*co*-13mol% 3HHx-*co*-27mol% 3H5HE)





Identification of terminal unsaturated bond reductase





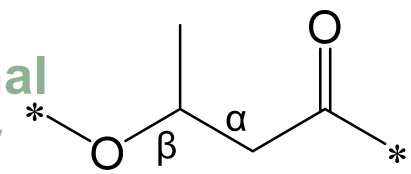
Recombinant enzyme by *E. coli* host

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

E1 Base materials for installing switching functions

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

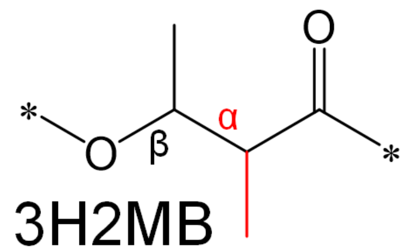
Conventional monomer



3HB

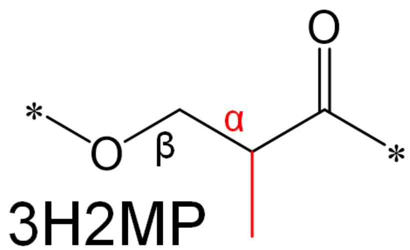
3-Hydroxybutanoic acid

Methylated at α-position monomer



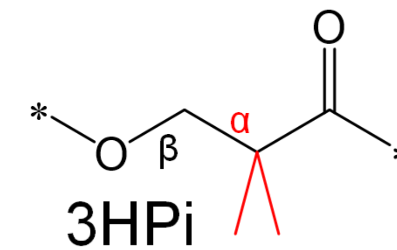
3H2MB

3-Hydroxy-2-methyl butanoic acid



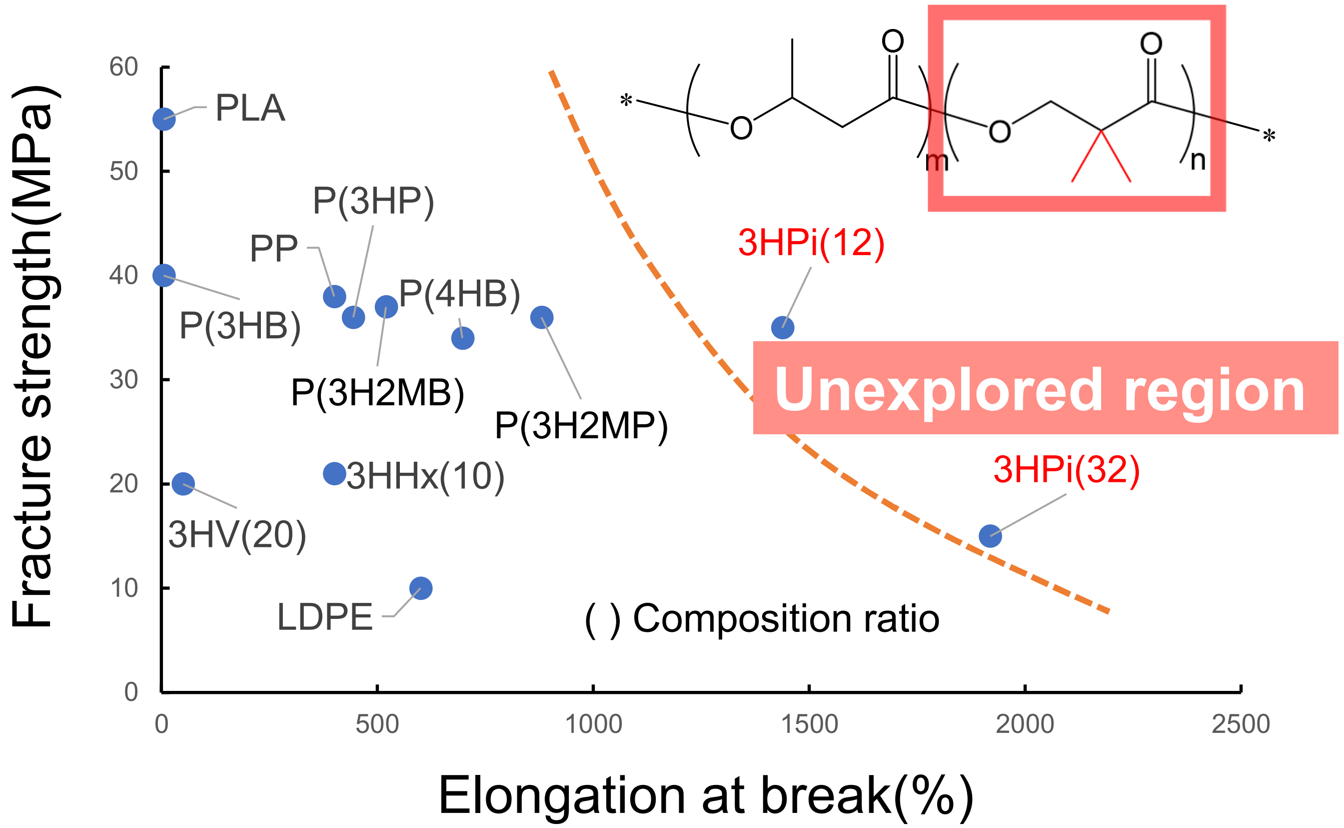
3H2MP

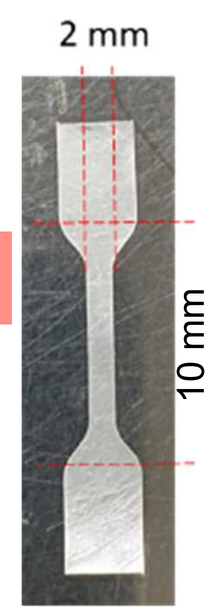
3-Hydroxy-2-methyl propionic acid



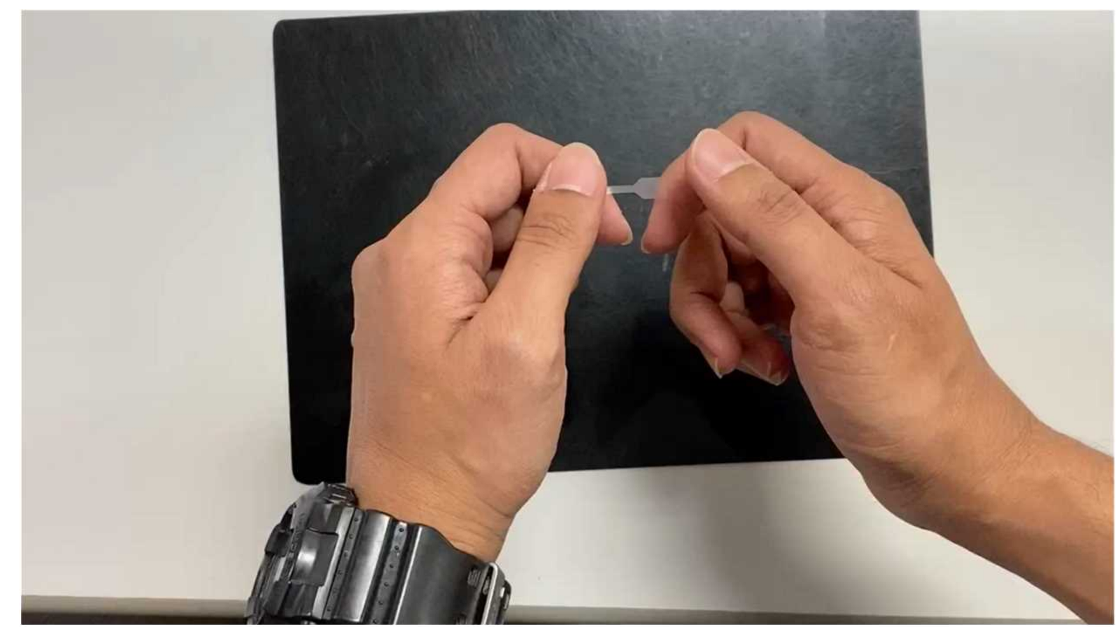
3HPi

3-Hydroxypivalic acid





Development of tough materials



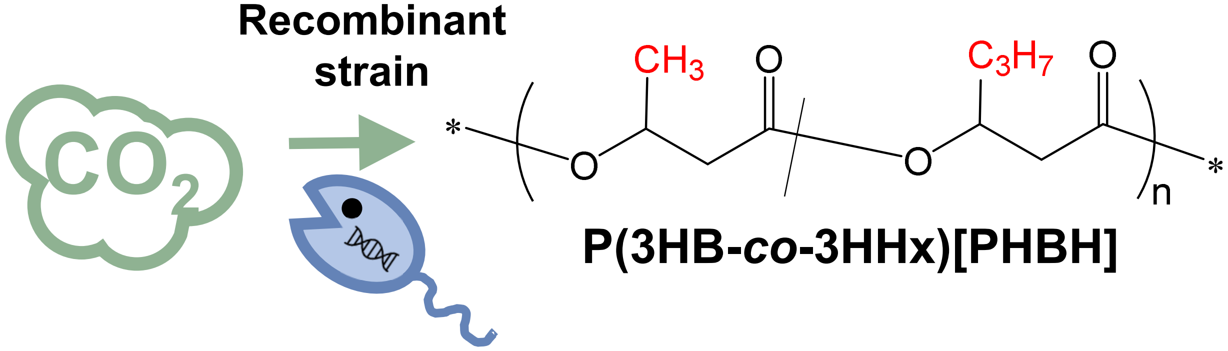
P(3HB-*co*-12 mol% 3HPi)

P(3HB-*co*-**X**)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	σ (MPa)	ε (%)	E (MPa)	Toughness (MJ/m³)
12 mol% 3HPi	5	61,134	28	35	1438	306	**306**
32 mol% 3HPi	1	57	3	15	**1919**	219	150

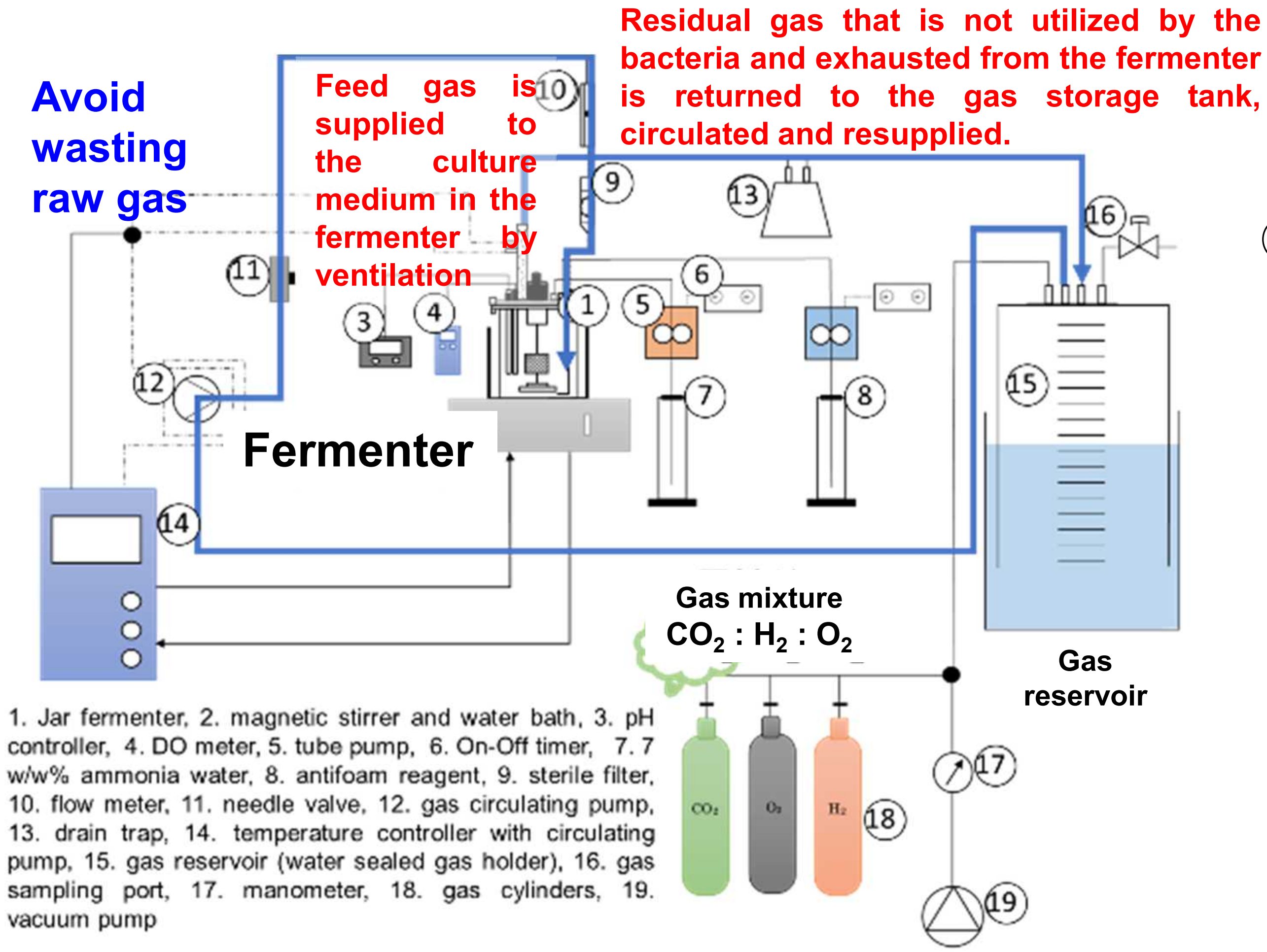
3HPi： 3-Hydroxypivalic acid

E1 Base materials for installing switching functions

- 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.



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



Closed circulation gas culture system

① Evaluation of each of 4 PHBH accumulating recombinant strains, selection of good strains

② Production of high PHBH concentration by jar culture of *C.necator MF01/JAc strain*

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
71.0	58.4	86.2	13.8	119	0.594
98.2	81.5	86.2	13.8	142.5	0.689

(2021)

③ Improved PHBH productivity

- Inorganic salts suitable for PHBH biosynthesis exploration of medium composition
- Improvement of fermenter agitation performance (raw material gas dissolution rate)

【Improvement of culture equipment and methods ①】 (2022)

- Maintains low concentrations of inorganic nutrients
- Mass flow controller continuously replenishes feedstock gas with constant composition

【Improvement of culture equipment and methods ②】 (2023)

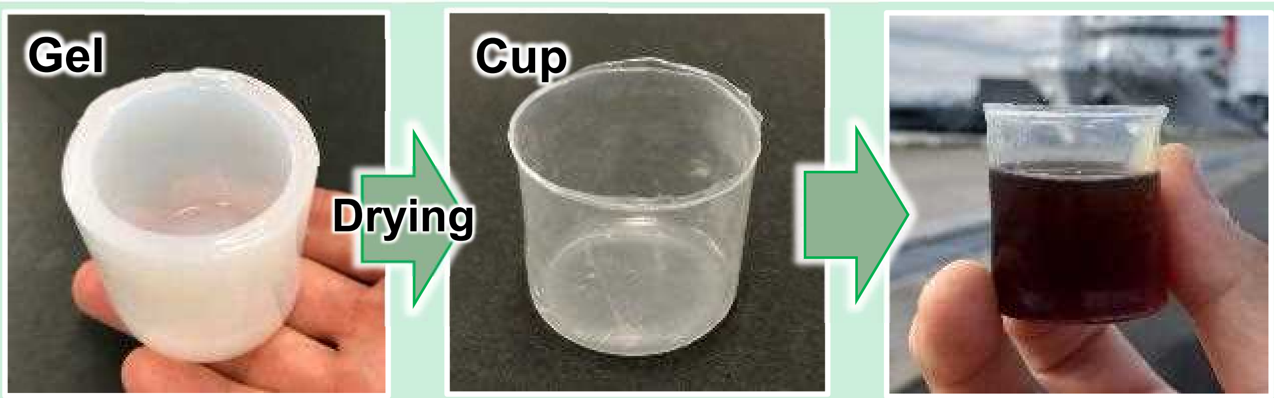
World Record: High polyester production from CO_2 including P(3HB)

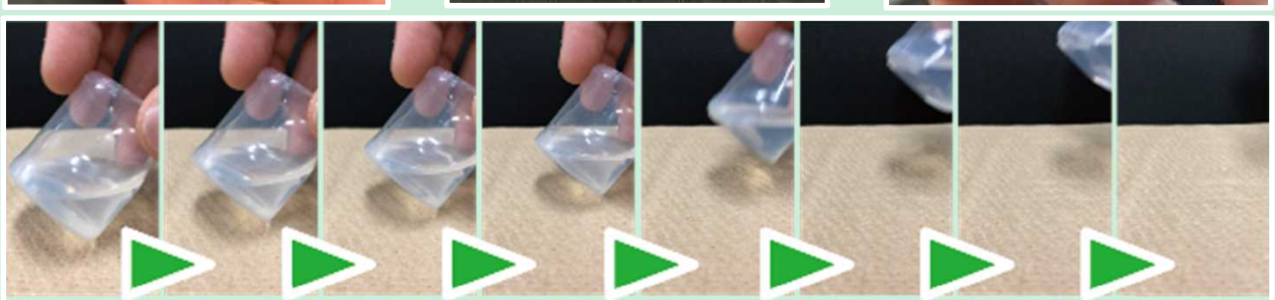
E1 Marine biodegradable plastics produced from polysaccharides

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

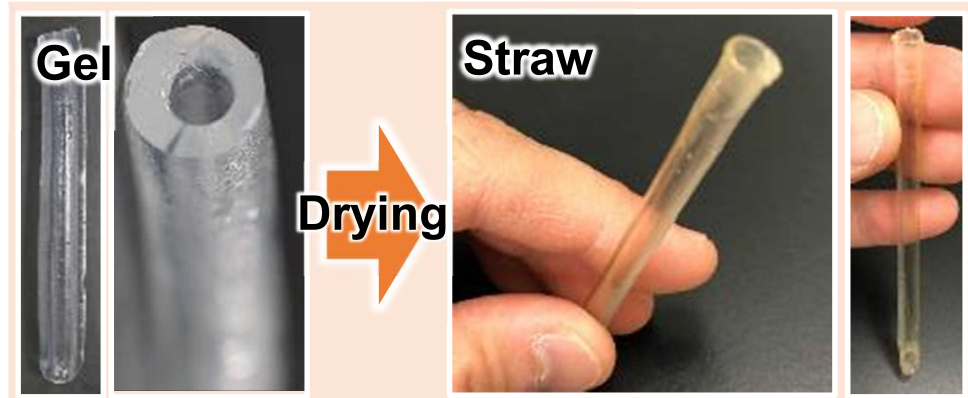
Compositionally identical with paper but more functional.

A transparent cup made of cellulose

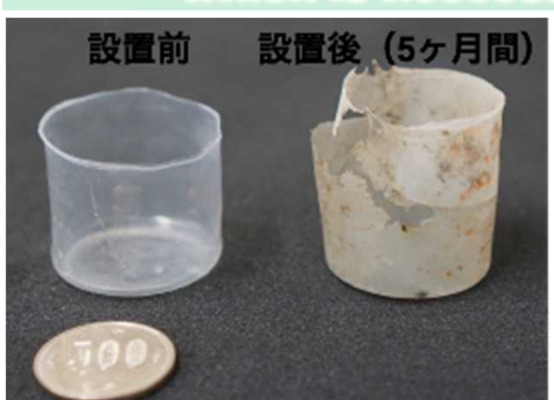


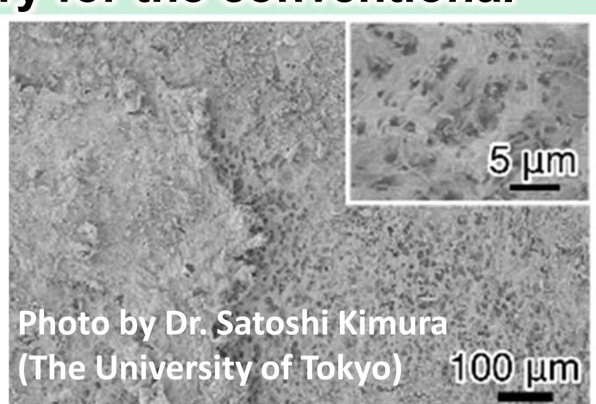


The cup holds water without inner film, which is necessary for the conventional



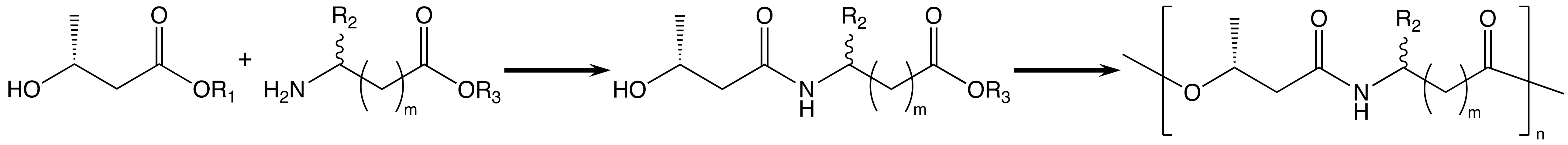
The brittleness of the material, which had been a problem, was overcome by improving molding and processing techniques, and we succeeded in preparing a complex-shaped component composed entirely of chitin.





- 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.

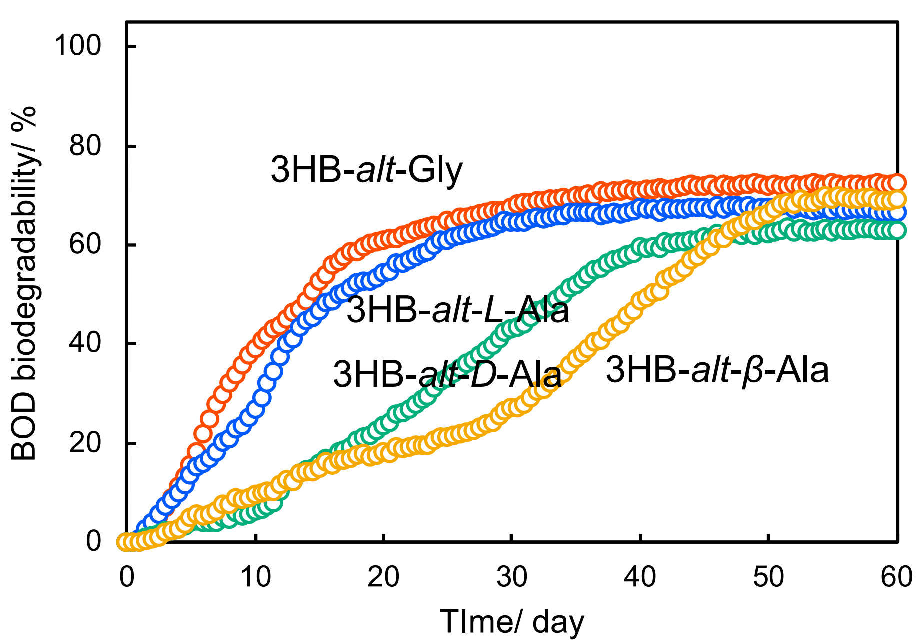
E1 Development of biodegradable polyamide



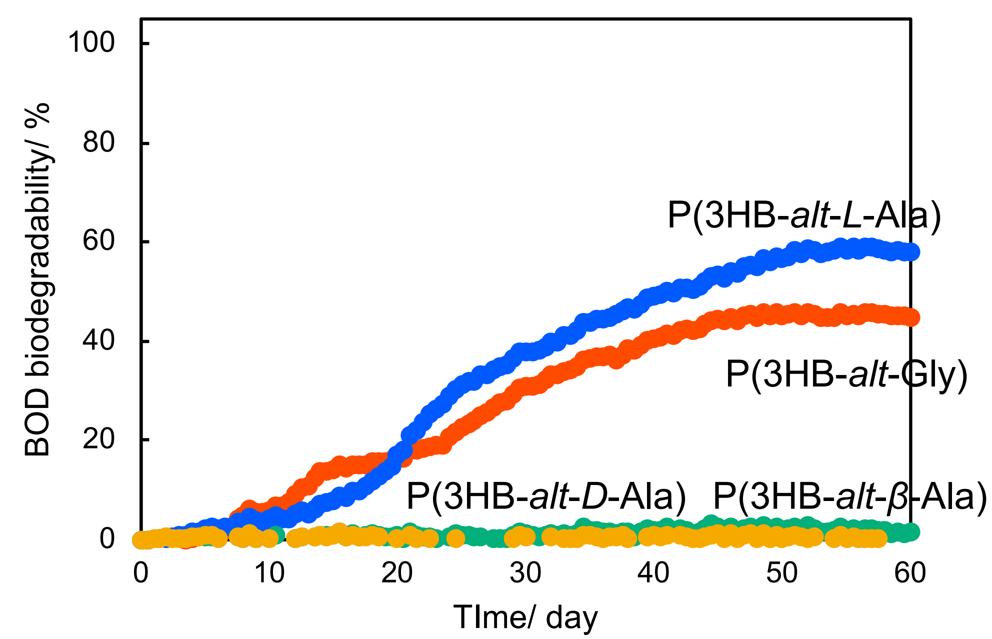
3-Hydroxybutanoic acid **Aminocarboxylic acid** **Dimer monomer** **Ester amide alternating copolymer**

	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

Feedstock: dimeric monomers

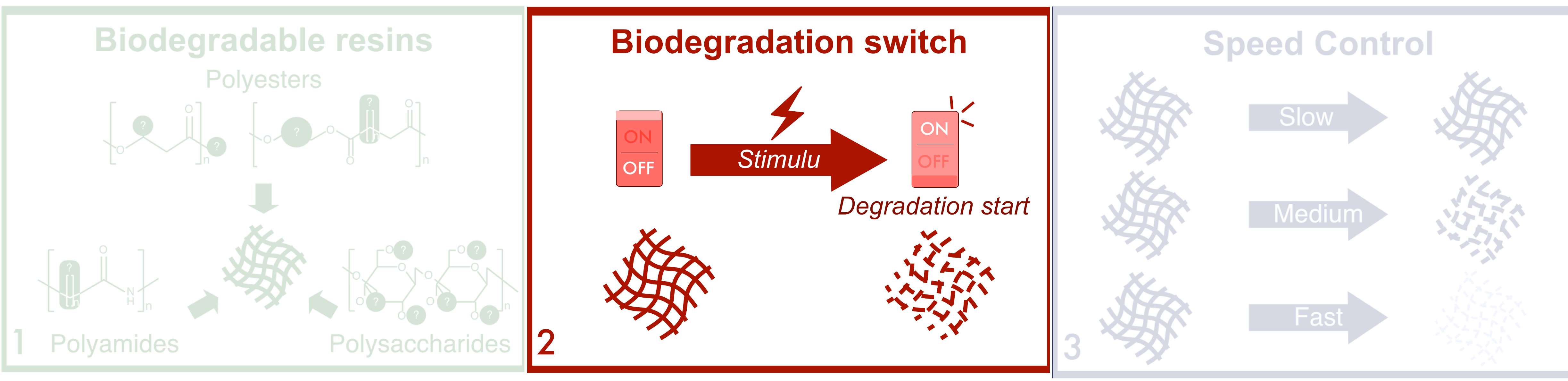


High molecular weight polyester-amide



8. Major results regarding ongoing topics

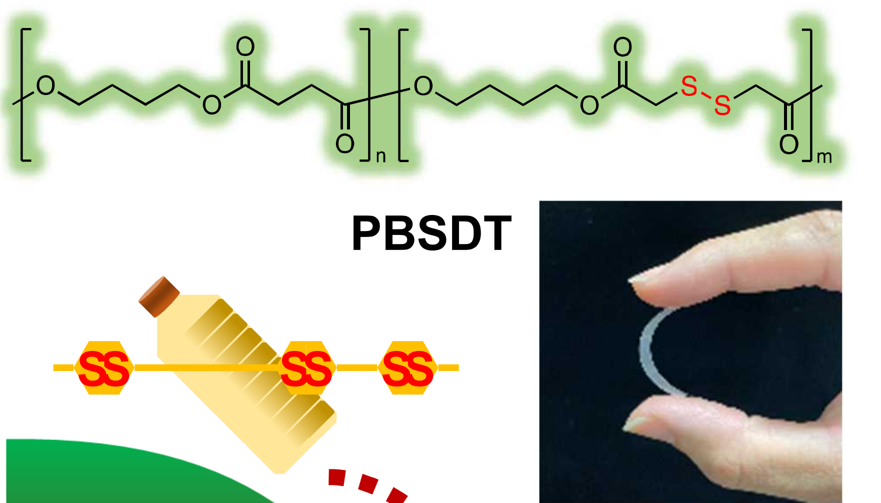
E2, Development of switching function to start biodegradation

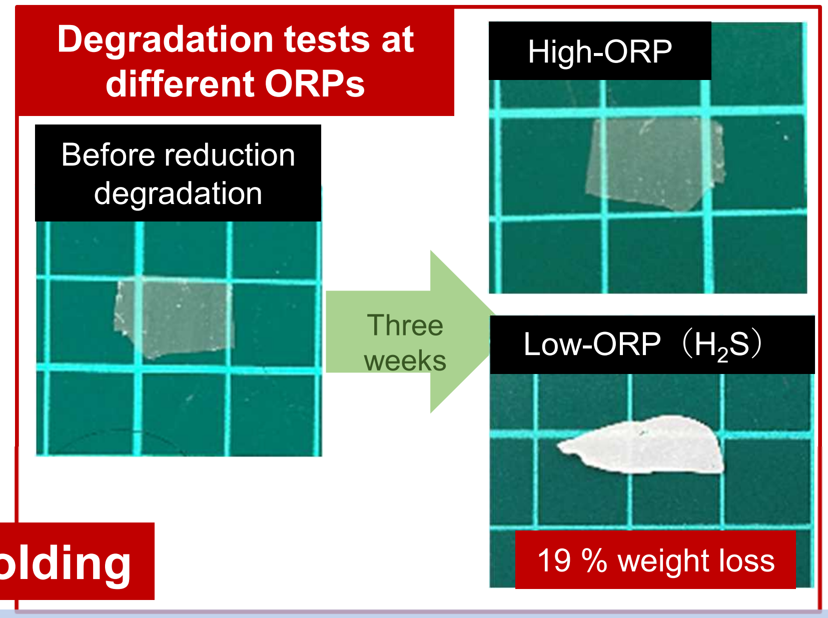


E2：	Development of switching function	pH Salt conc. ORP Pressure Temperature Wearing

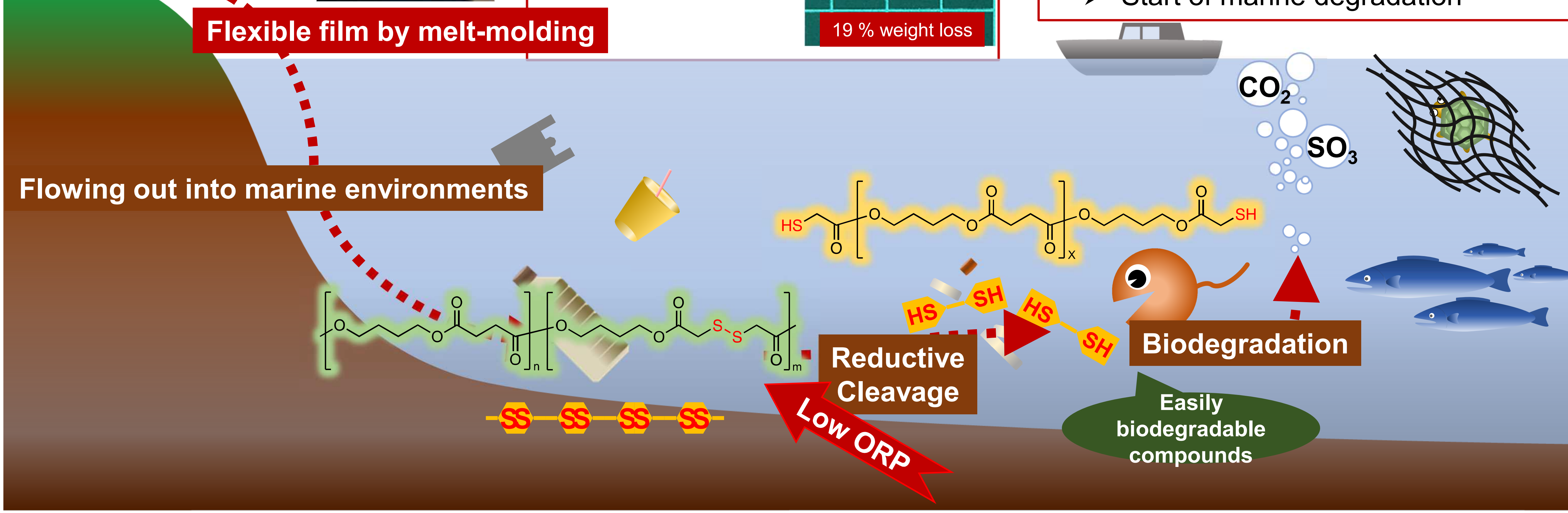
E2 Switching triggered by difference in ORP

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

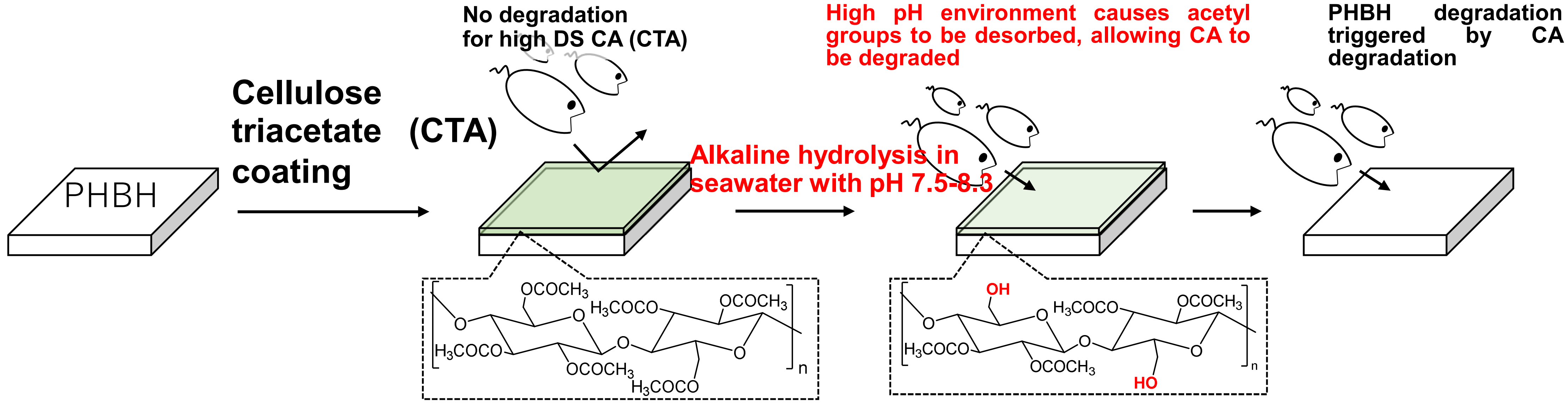




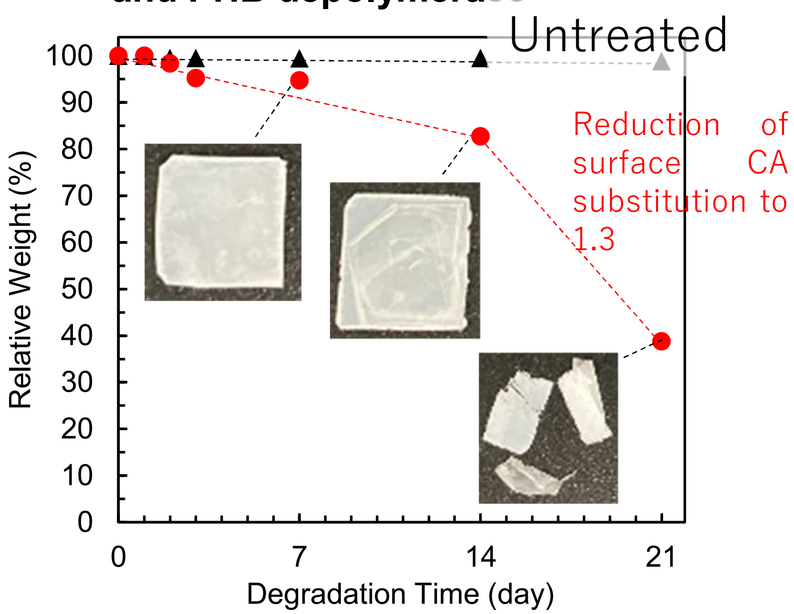
- PBSDT Physical Properties
  - PBSA analogues
  - Can be formed into films
  - Can be synthesized from commercial reagents
- Normal seawater = non-degradable
- Seabed (reducing environment)
  - Disulfide bond cleavage
  - Start of marine degradation

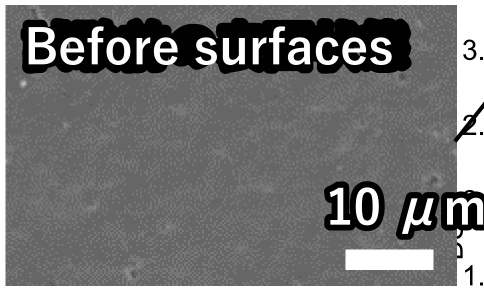


E2 Switching triggered by difference in pH

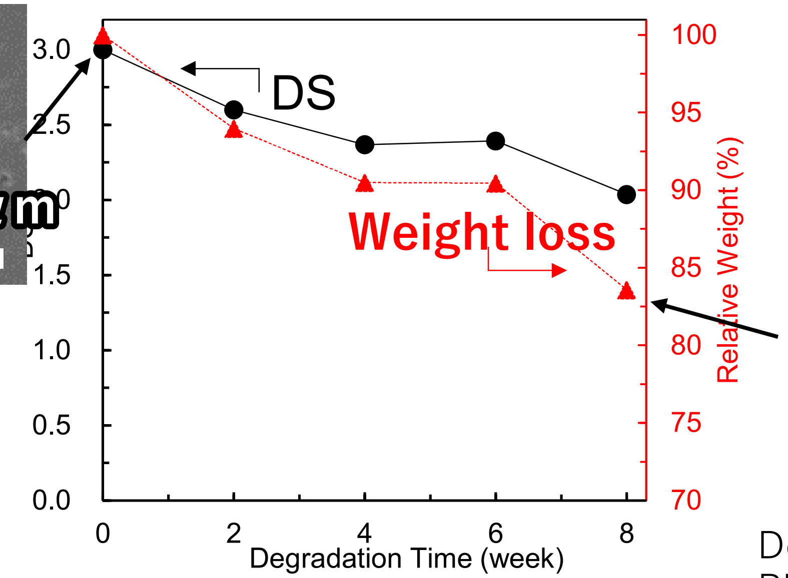


Enzymatic hydrolysis cellulase and PHB depolymerase

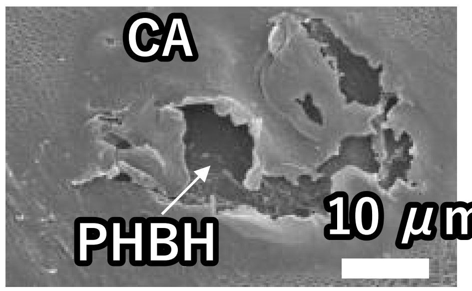




Degradation test in seawater

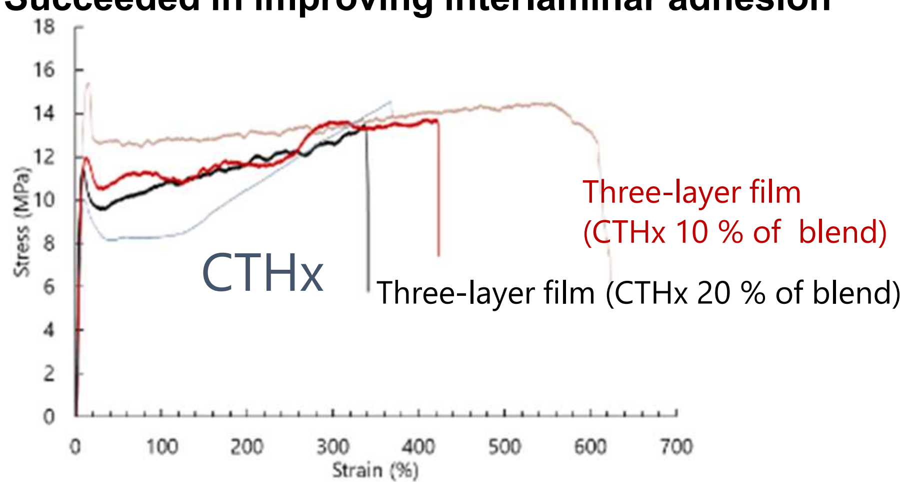


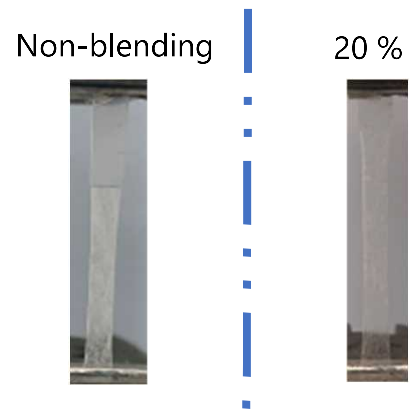
8 weeks later



Degradation of CA exposes PHBH

Succeeded in improving interlaminar adhesion

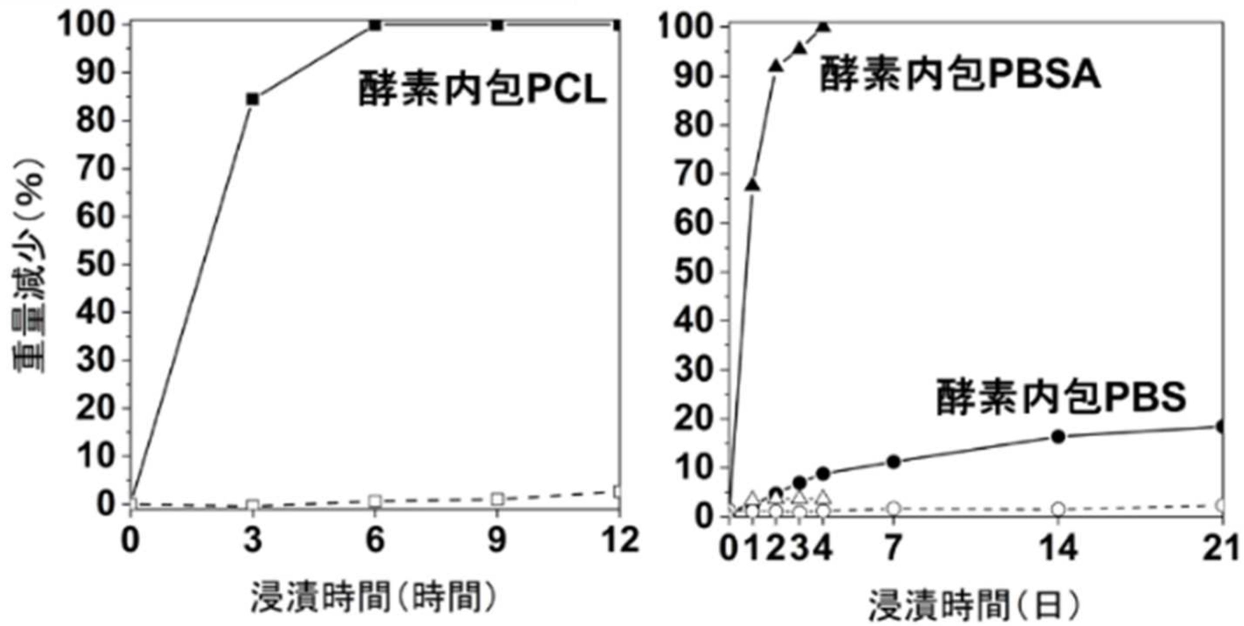


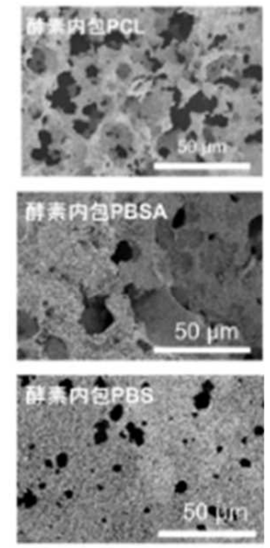


Base resin properties are expressed through improved adhesion

E2 Switching triggered by wear (Enzyme)

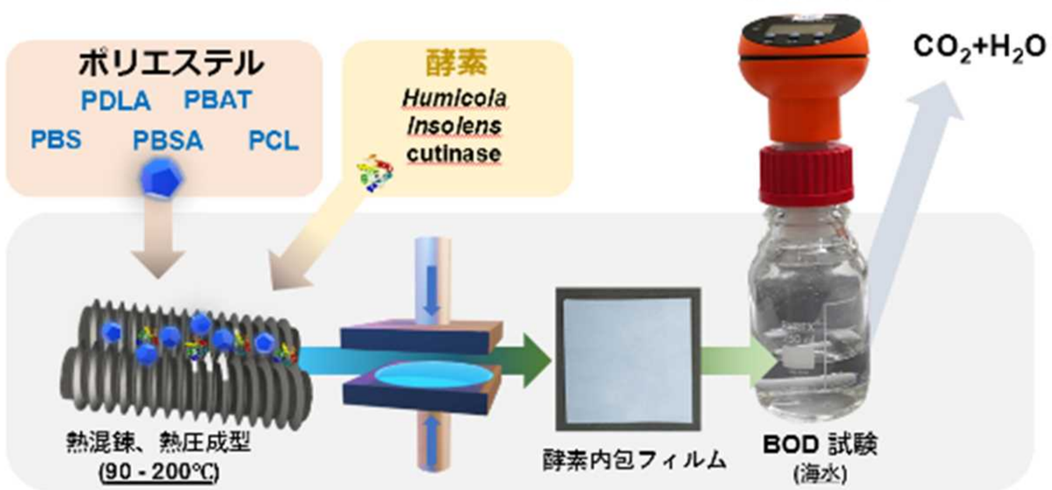
Degradation of CaLB lipase-incorporated PCL, PBS, and PBSA in water

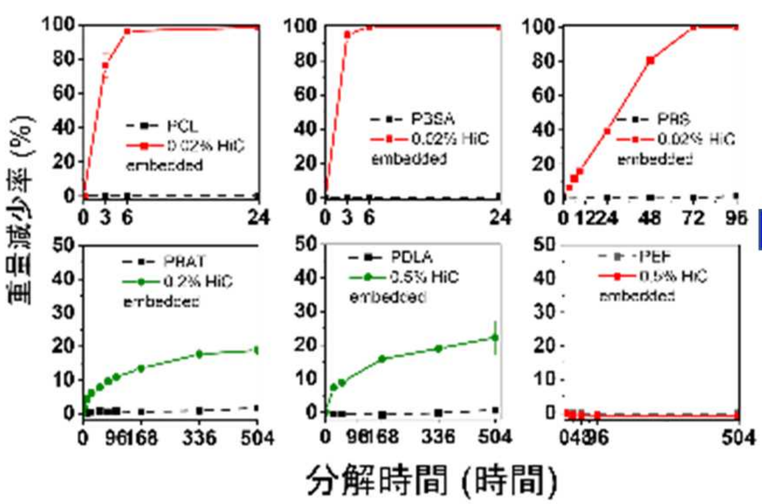




- PCL, PBS, and PBSA also succeeded in making enzyme-embedded plastics by melt blending.
- PCL completely degraded in 6 hours and PBSA in 3 days.
- PBS degrades 20% in 21 days.

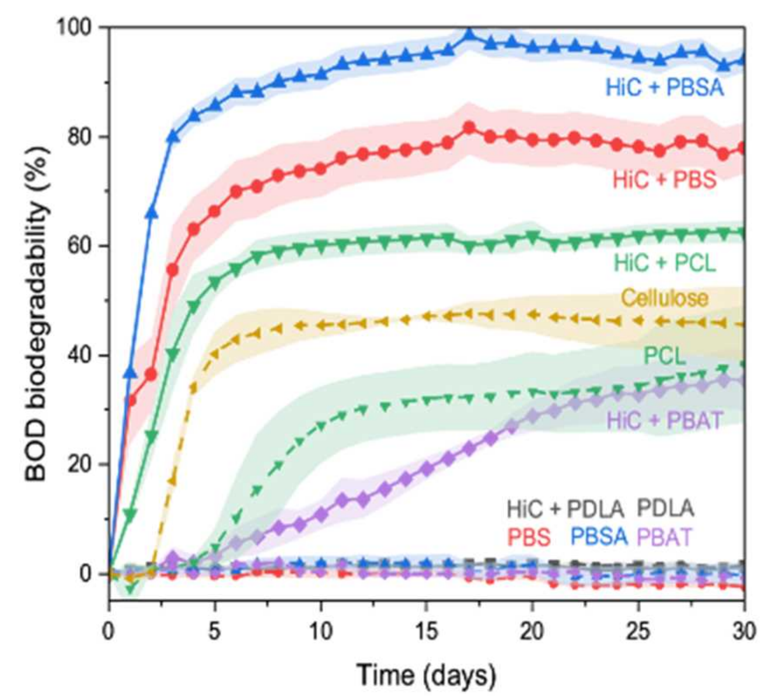
Development of enzyme (lipase) embedded biodegradable plastics





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

Successfully reduced degradation time

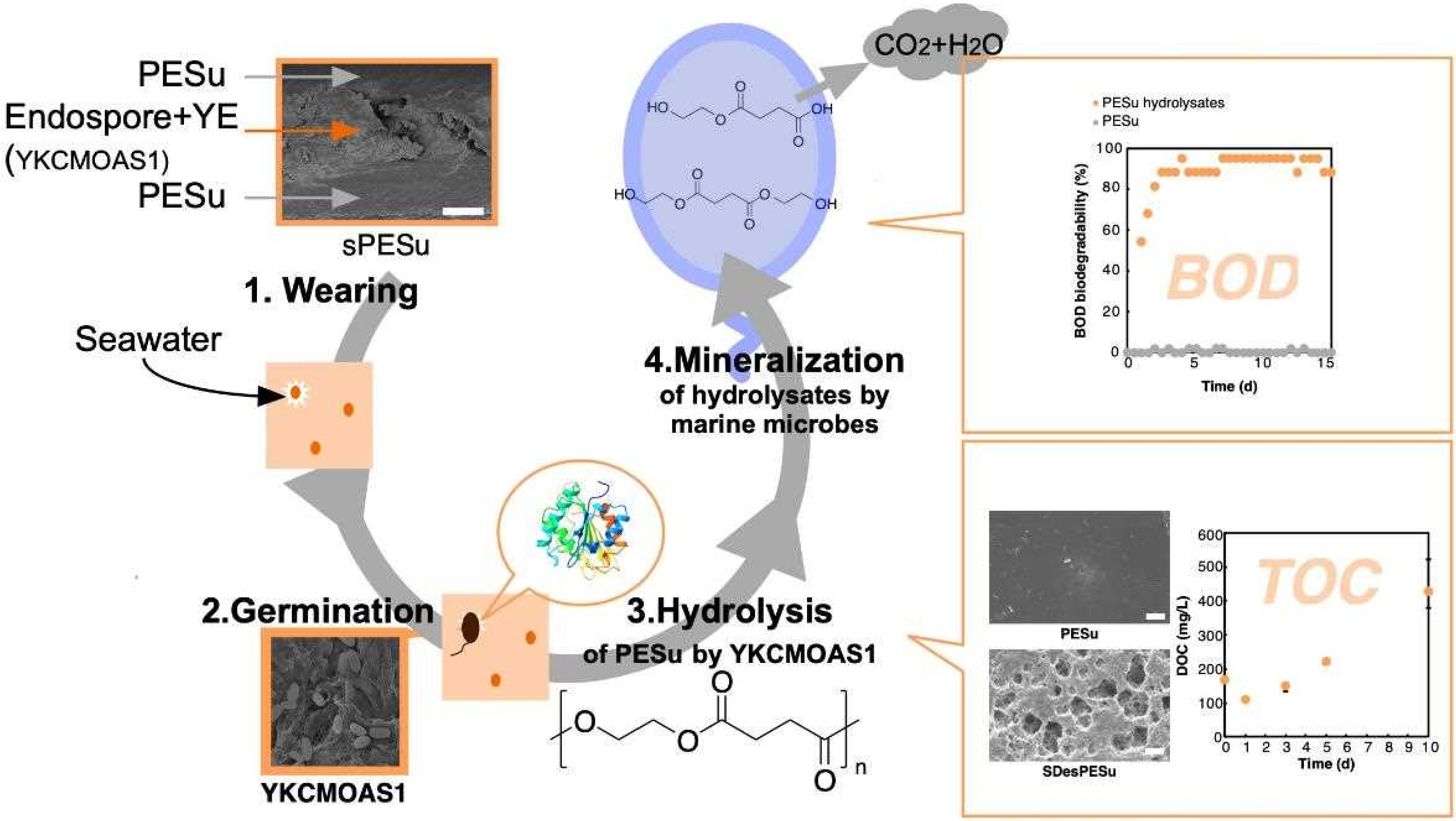


BOD biodegradability curves of various biodegradable plastics melt-mixed with cutinases in seawater of Tokyo Bay

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

E2 Switching triggered by wear (Enzyme)

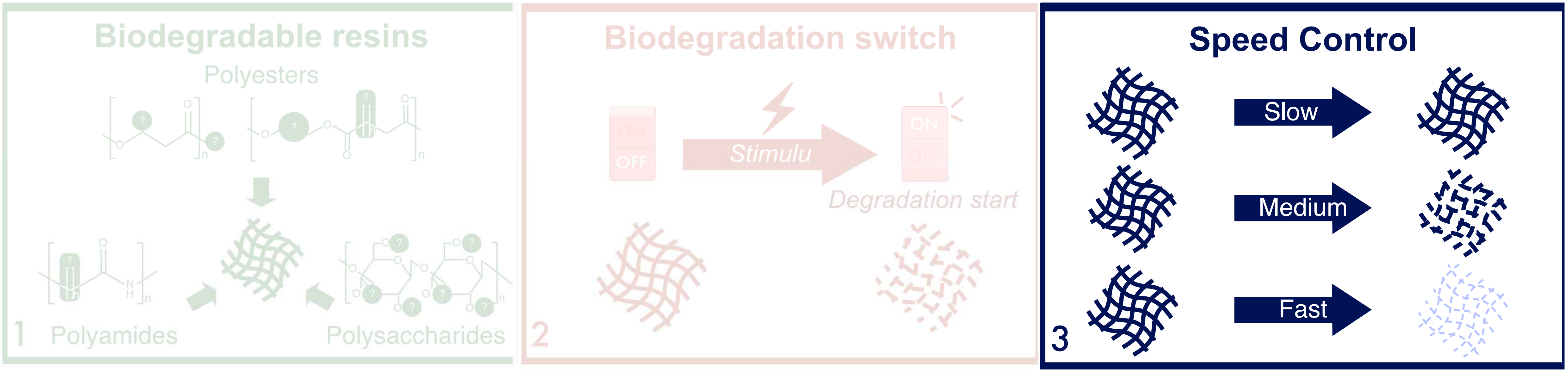
Degradation is triggered by wear, and biodegradation proceeds as endospores transform to vegetative cells.



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

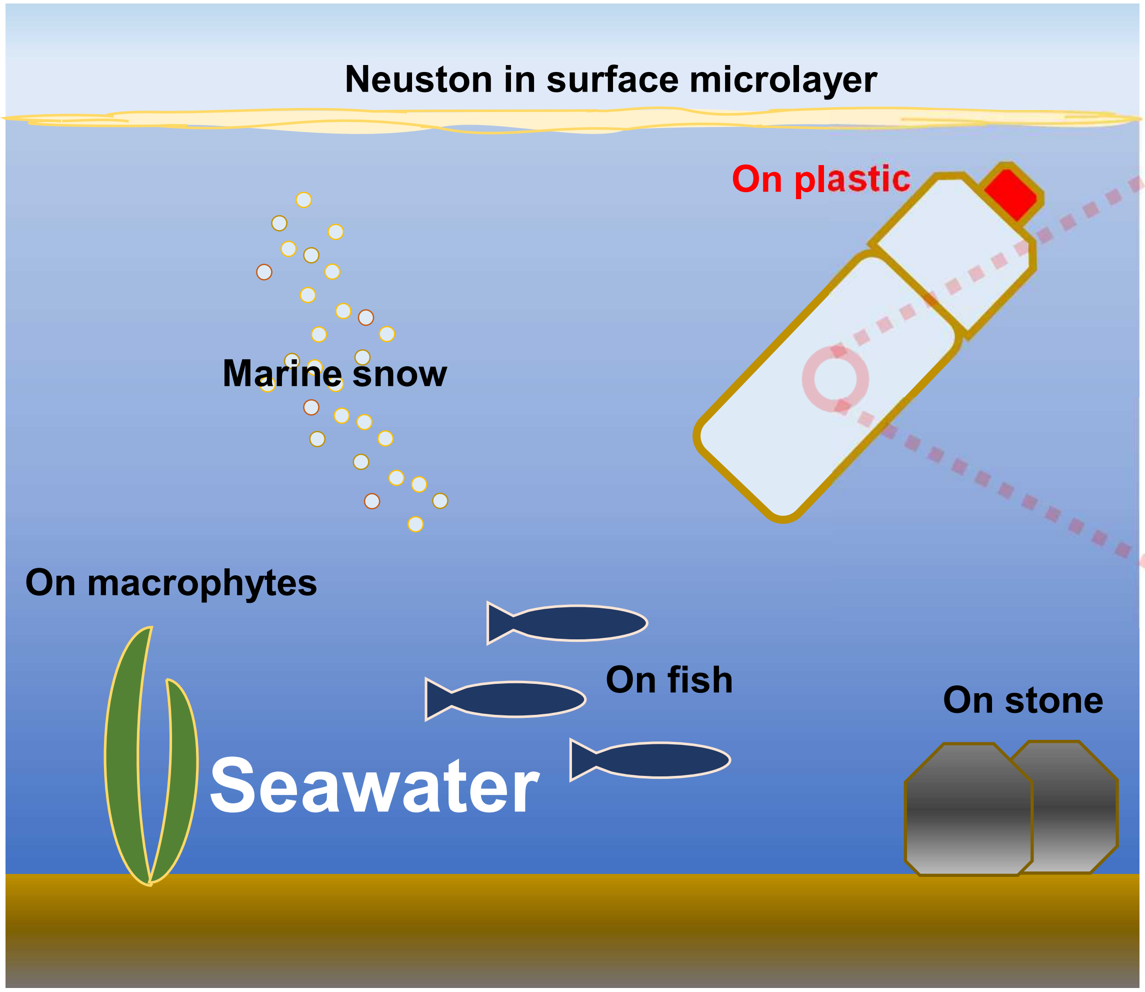
E3 8. Major results regarding ongoing topics

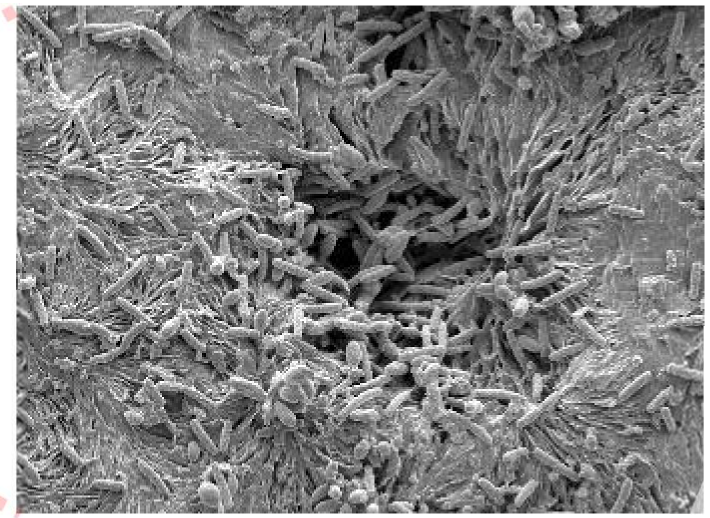
E3, Biodegradation rate control



E3：	Biodegradation rate control	Acceleration	Biological factor
			Material science
		Deceleration	Material science

E3 Plastisphere：Microbial flora formed on plastic surface

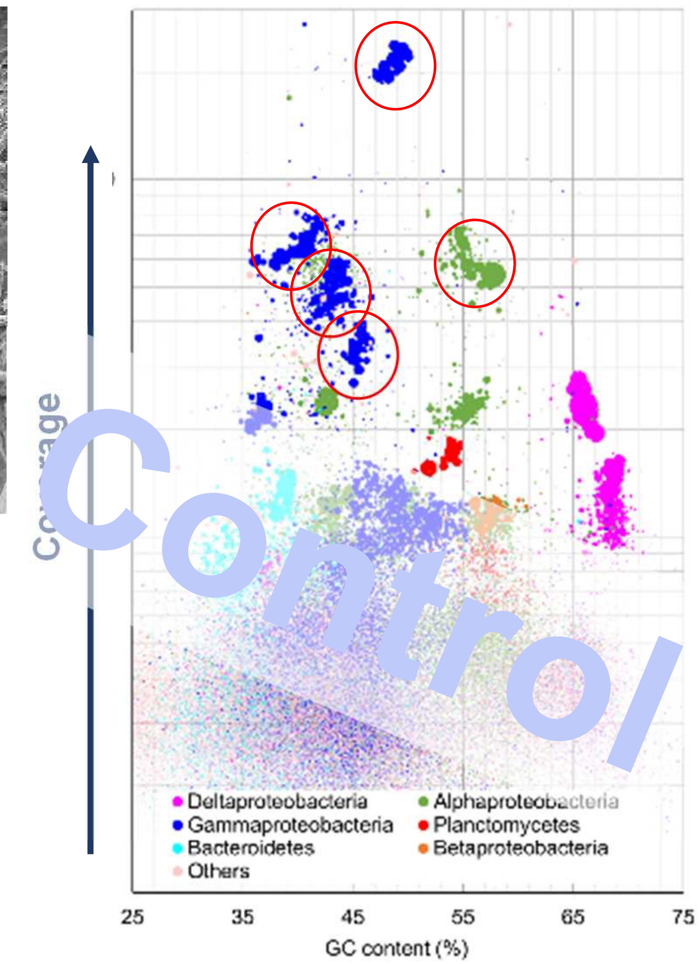




SEM image of plastisphere formed on the biodegradable plastic surface.

Metagenome analysis

Microbial accumulation in the plastisphere



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

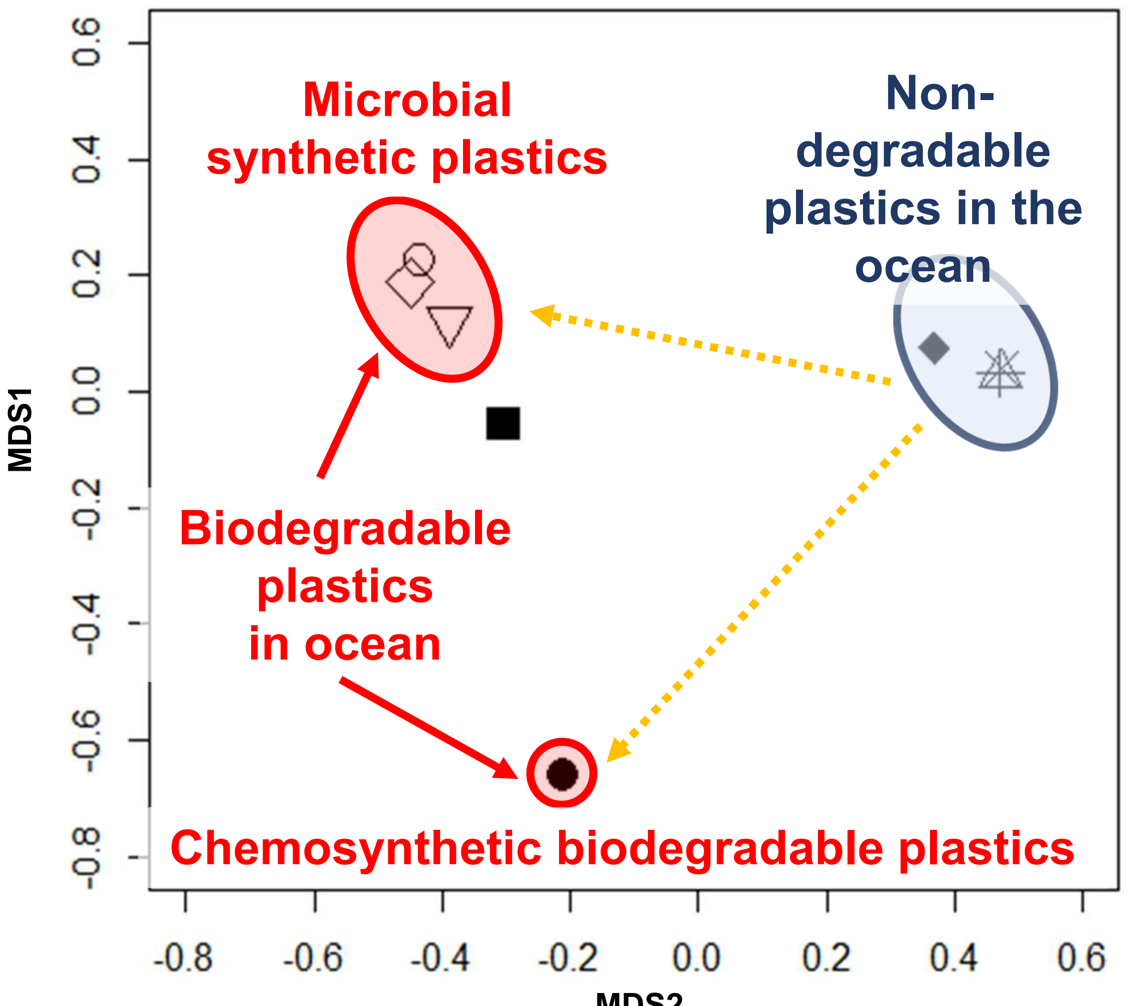
Elucidate the biodegradation mechanism of plastic and control the degradation rate.

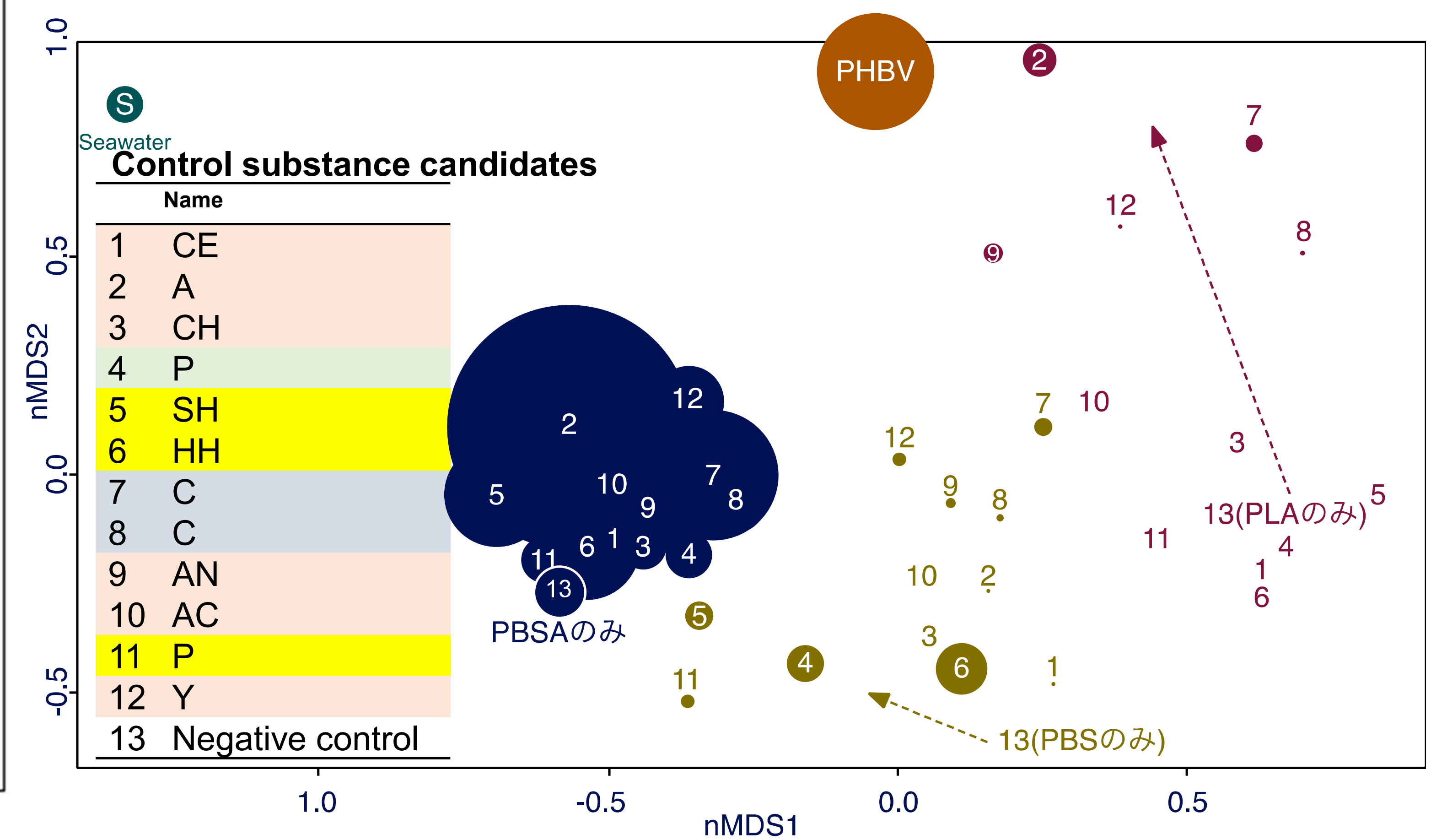
Also investigate the plastisphere in non-oceanic environments.

Biodegradable plastics is exposed to pond.

E3 Biodegradation rate control by controlling plastisphere

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.





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

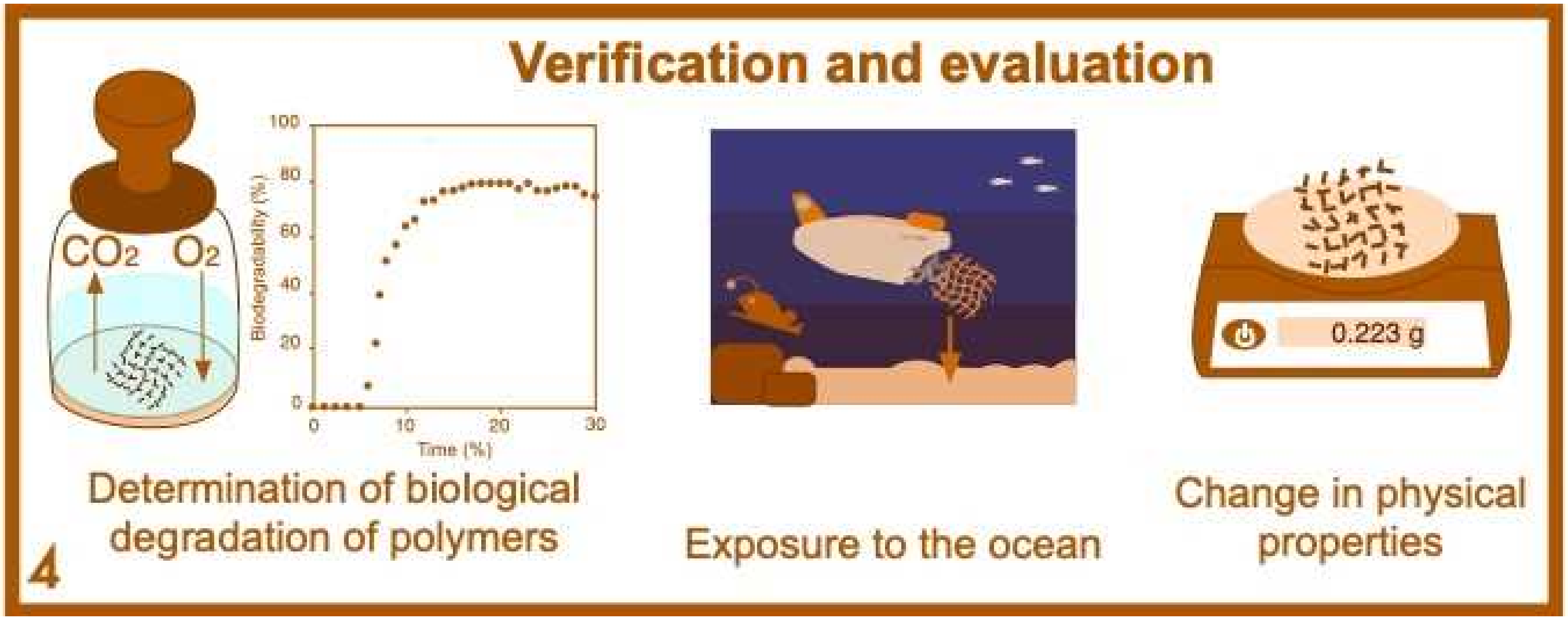
Non-metric multidimensional scaling (nMDS) based on the Bray-Curtis index. Numbers in the plot indicate the type of substance. The area of the plot shows the biodegradation rate except for seawater.

PBSA
No.2、No.5、No.6、No.7
PBSu
No.5、No.6 – also gave BOD-biodegradability for PBSu.
PLA
No.2、No.7、No.9
Effect on increase in the degradation rate.

E4

8. Major results regarding ongoing topics

E4, Validation and evaluation of biodegradability in laboratory and deep-sea environments



E4： **Verification and evaluation of marine biodegradability**	**In vitro**	BOD biodegradability Test condition
	In vivo	In Shallow water In deep-sea Buoy

E4 Biodegradation tests of novel materials

Types of marine biodegradation tests

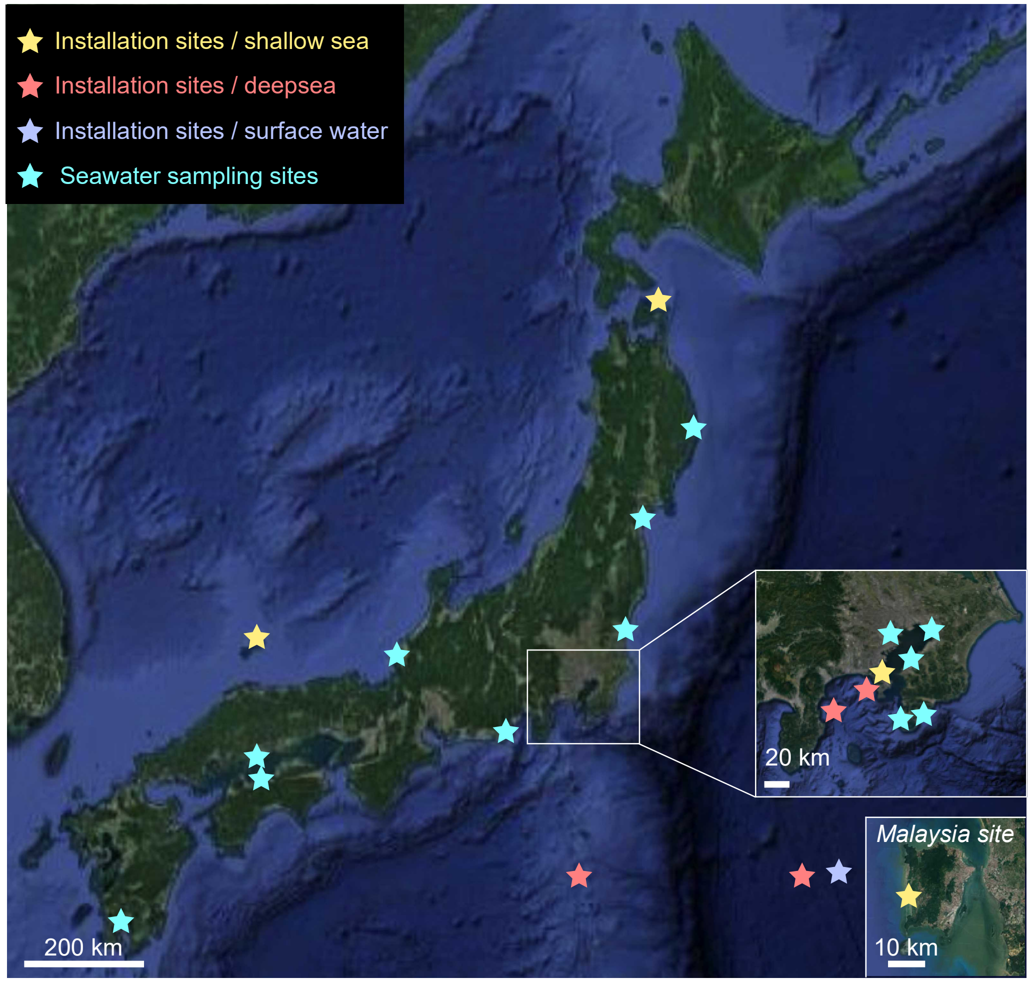
- ***In vitro***
  - Normal pressure (water tank, BOD)
  - Pressurized (pressurized vessel)
- ***In situ***
  - Shallow water
  - Deepsea water
  - Pelagic surface environment

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"

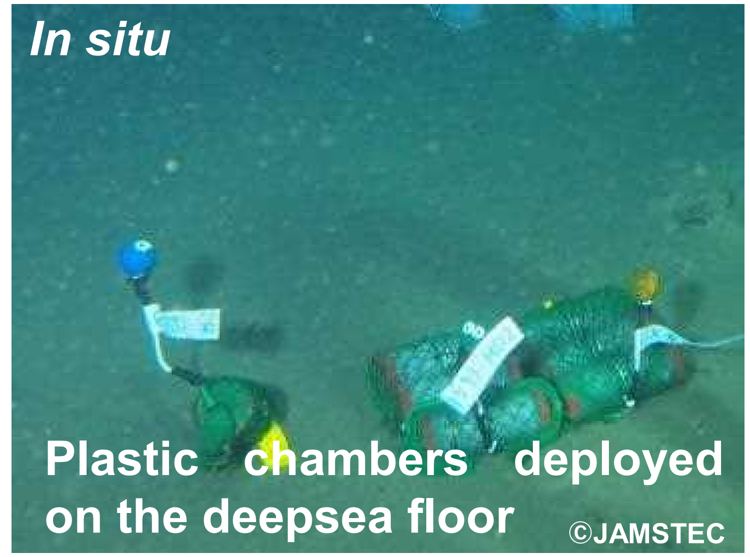
- **Biodegradation assessments in the Deep Sea and Information Exchanges**
  - **Conducting experiments in the deep sea using the Shinkai 6500**
  - **Promotion committee meetings and joint workshops held (4 times / year)**

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)**

E4 Biodegradation tests of novel materials

Deployment and recovery with the manned submersible



Plastic chambers deployed on the deepsea floor

Sample installation at Mutsu Lab, Aomori Pref

Between 2020 and 2022, six 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.

Degradability testing began in Dec 2023 at the Mutsu Lab.

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

Oki Coastal Lab., Shimane University

Immersion tests are scheduled to begin in February 2024 at the Marine center, Kyoto Pref.

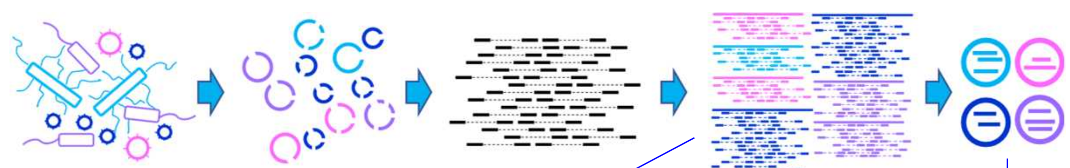
Immersion tests began in October 2023 at the Oki Coastal Research Lab, Shimane University.

Sample installation at Oki

Free-flowing water tank

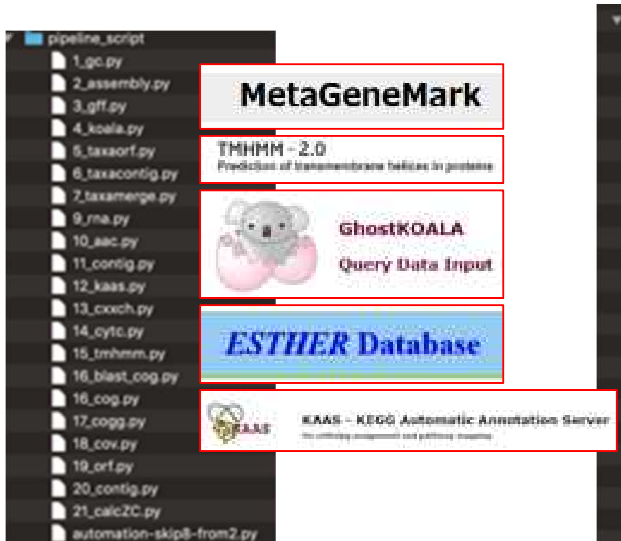
E4 Meta-omics analysis of plastisphere correlating with biodegradation

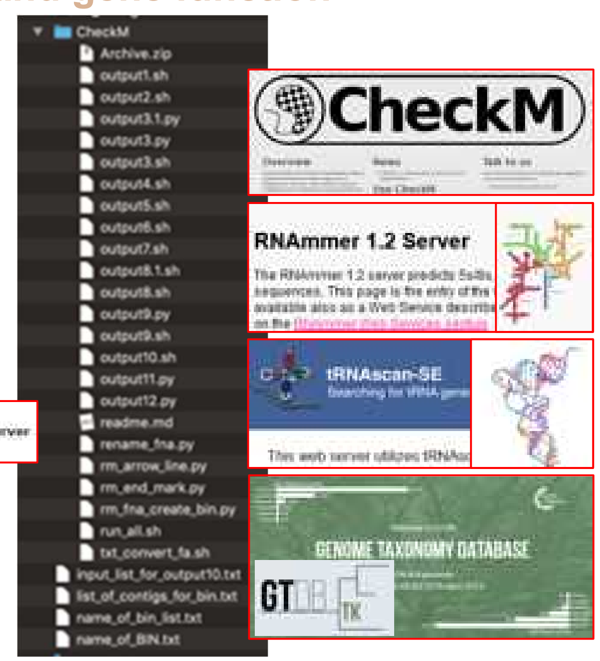
Pipeline



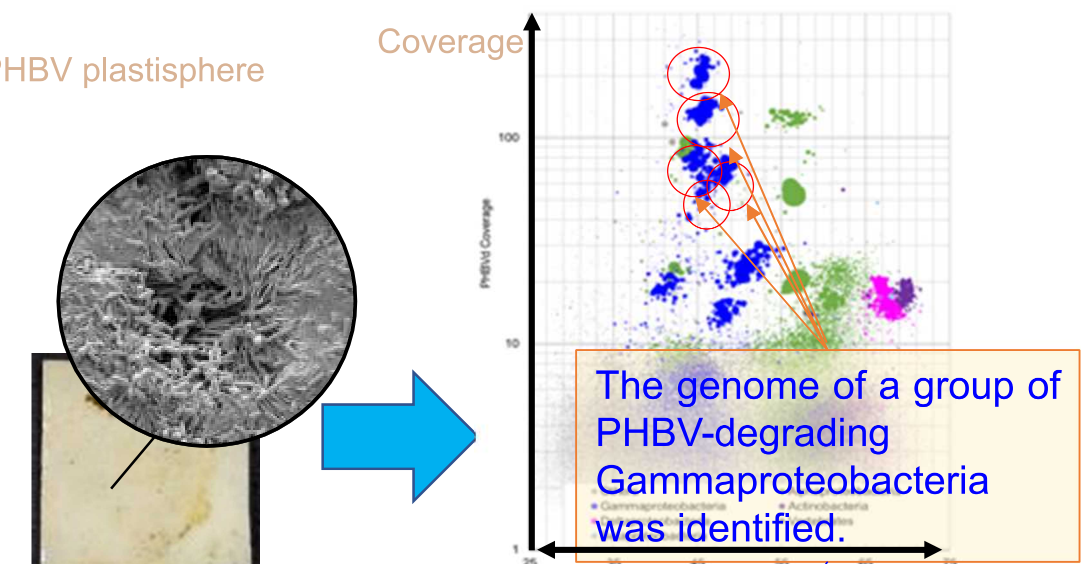
Pipeline for genome quality control

Pipeline for analysis the genome and gene function





Metagenome analysis of plastisphere



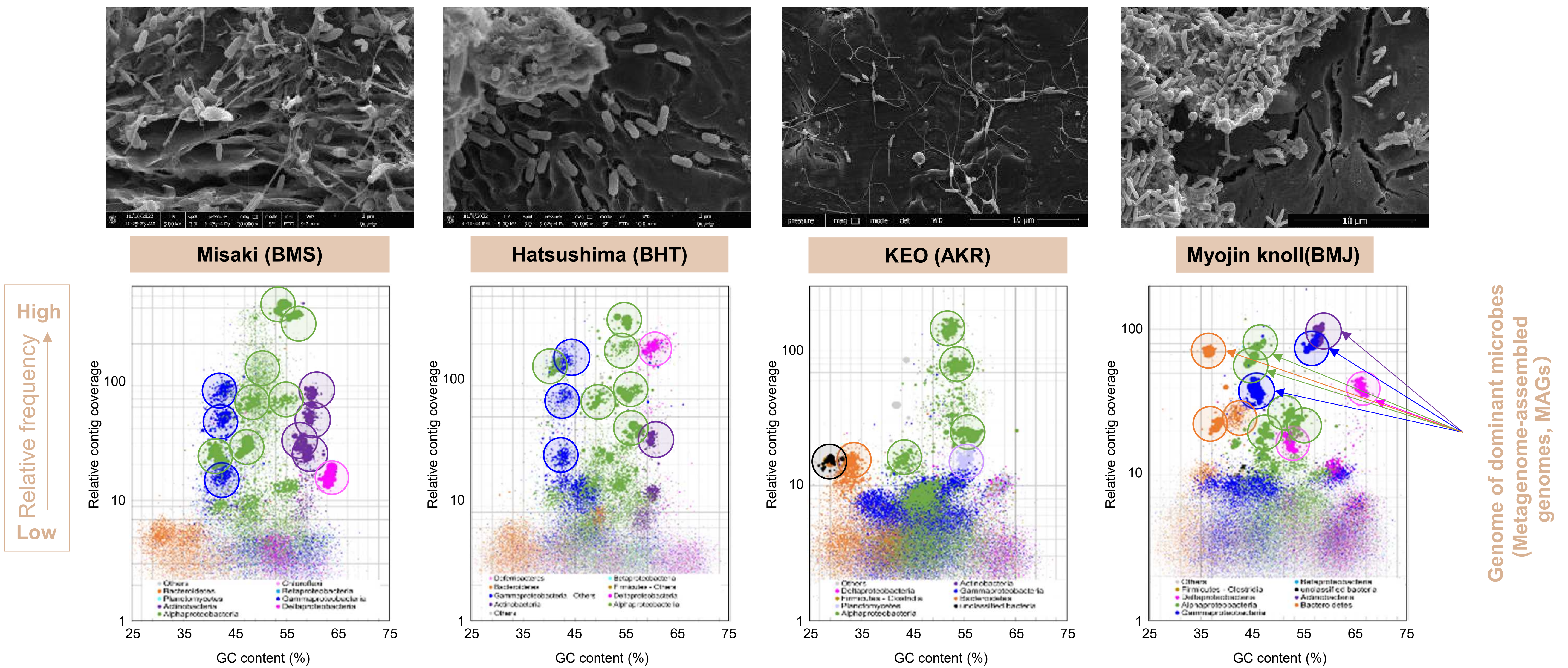
Function	ESTHER ID	Gamm1	Gamm2	Gamm3	Gamm4	Gamm6	Gamm7	Gamm8	Gamm5	Gamm9	Gamm10	Alph1	Alph2	Alph3	Alph4	Alph6	Acti1	Delt1	Alph5
Esterase_phb	Esterase_phb	23	13	19	20	18	14	1	0	3	1	0	0	0	0	0	1	6	0
Abhydrolase_6	PHB_depolymerase_PhaZ	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0
Esterase_phb_PHAZ	Esterase_phb_PHAZ	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Thioesterase	Thioesterase	9	9	4	4	8	8	13	5	0	0	1	0	0	2	2	6	10	0
Lipase_2	Lipase_2	10	2	6	10	11	5	0	1	0	0	0	0	0	0	0	0	1	0
Abhydrolase_6	6_AlphaBeta_hydrolase	15	12	12	18	19	14	14	32	11	15	9	6	16	32	24	50	31	0
Bacterial_lipase	Bacterial_lip_Faml.3	4	0	1	3	2	1	2	2	2	1	5	5	10	0	25	1	0	3
AlphaBeta_hydrolase	AlphaBeta_hydrolase	2	5	2	2	4	4	1	3	3	0	6	3	5	3	4	9	8	0
Hormone-sensitive_lipase_	Hormone-sensitive_lipase_like	1	0	1	2	1	4	0	1	8	1	2	0	1	9	7	12	6	0
Epoxide-hydrolase_like	Epoxide_hydrolase	2	2	1	2	1	1	1	0	5	0	0	0	1	3	3	10	5	0
Peptidase_S9	Prolyl_oligopeptidase_S9	3	1	3	4	3	0	2	10	0	1	0	0	0	4	0	0	3	2
Hydrolase_4	Proline_iminopeptidase	3	1	1	3	2	2	1	4	1	1	1	1	1	1	1	3	8	0
Dienelactone_hydrolase	Dienelactone_hydrolase	1	1	0	1	2	1	1	2	1	1	0	0	1	2	1	8	3	0

The genome code many PHB depolymerase genes

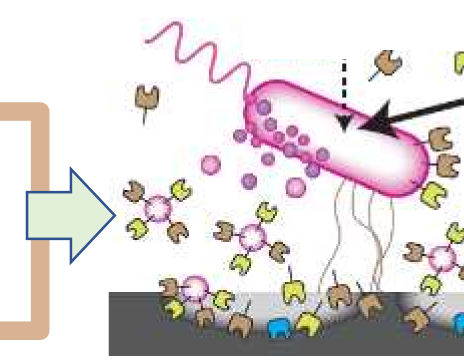
A method was developed to efficiently and rapidly analyze large amounts of data.

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

E4 Meta-omics analysis of plastisphere correlating with biodegradation



- ✓ 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**



E4 *In vitro* biodegradation tests of novel materials

Biodegradable plastics + O_2 ▶ CO_2

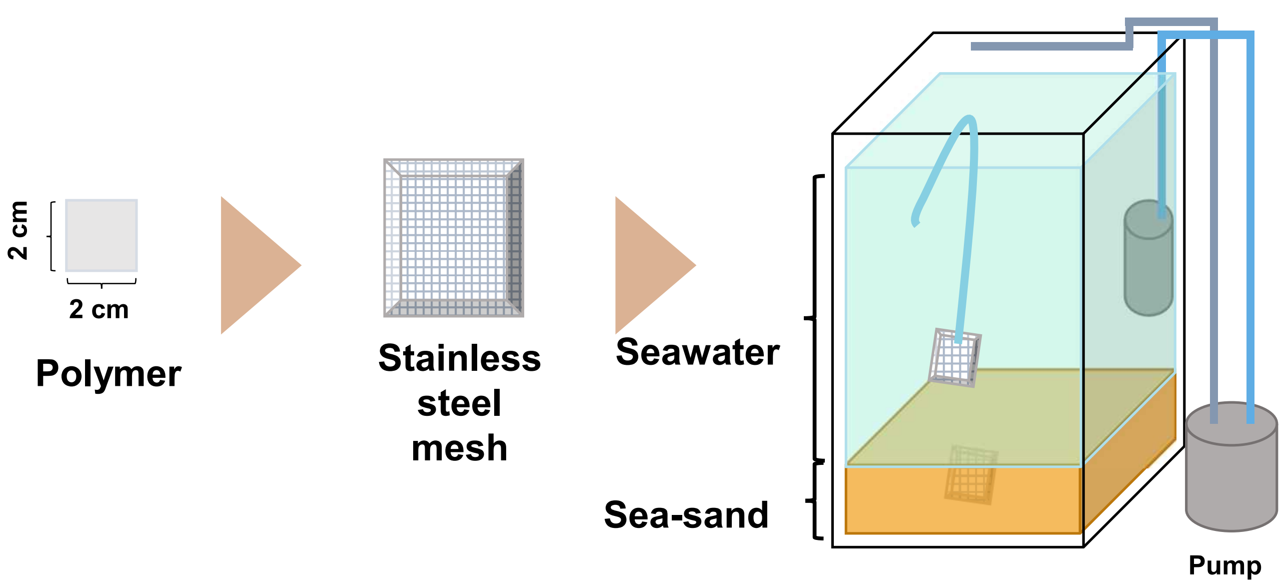
By microbes

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

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

ThOD: Theoretical oxygen demand

BOD biodegradation testing



Tank experiment

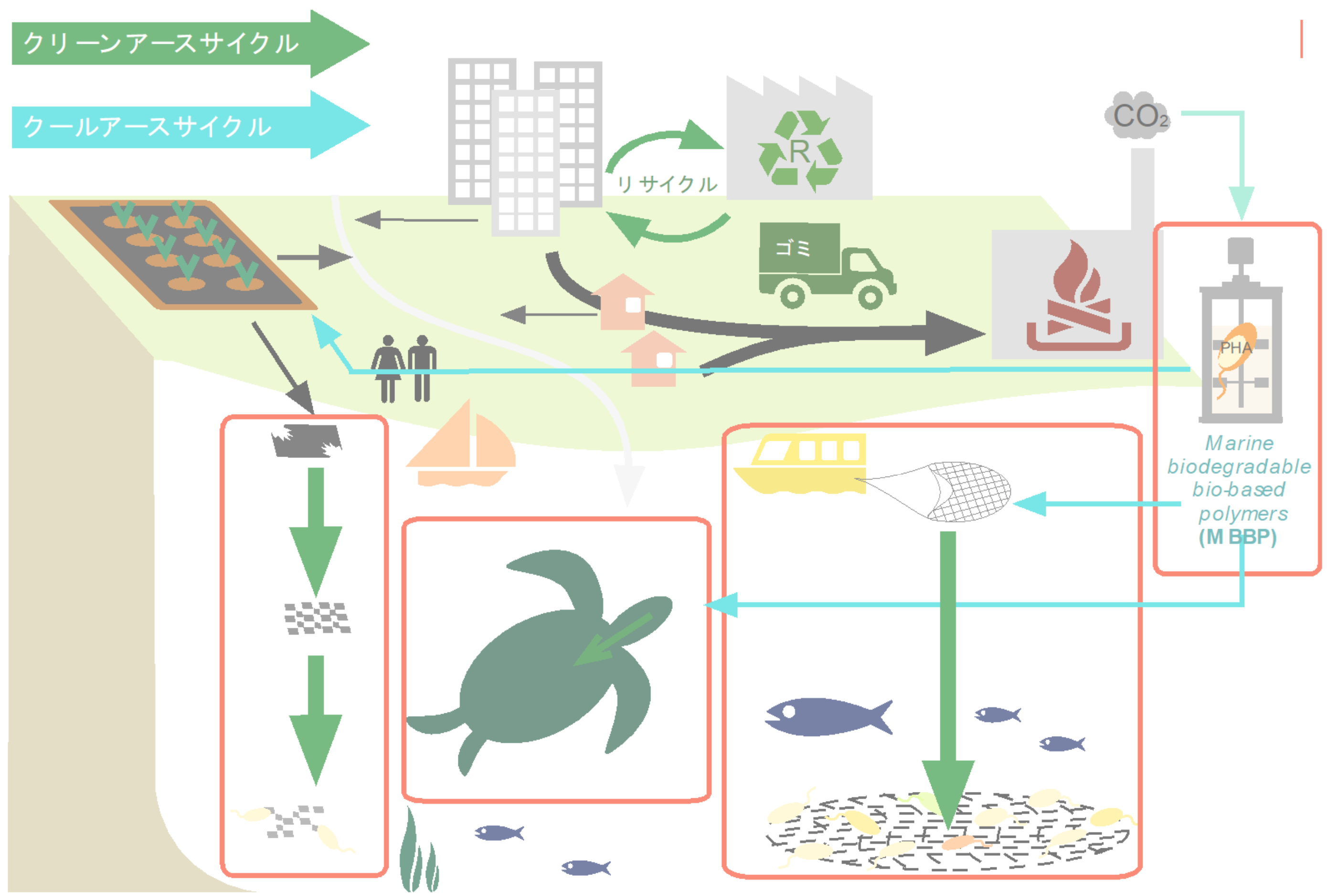
- Weight loss
- Morphology of surface
- Mechanical properties
- Plastisphere analysis

SI

8. Major results regarding ongoing topics

Research and development for social implementation (SI)

5. Social implementation of developed technologies



SI

R & D for social implementation

NISSHINBO

Controlling the biodegradation of plastics in the ocean by some compounds

＜ L compound addition concept ＞

In soil

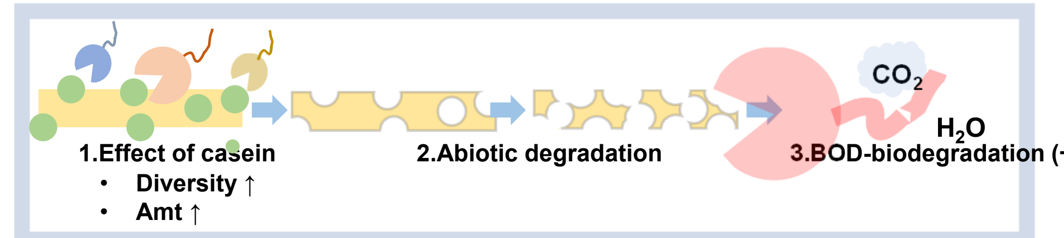
Large number of microbes

⇒ ***Fast biodegradation***

In seawater

Small number of microbes

⇒ ***Slow biodegradation***



Search for marine degradation accelerators that attract microorganisms and accelerate degradation

● Selection of L compounds

Effective utilization of food-waste

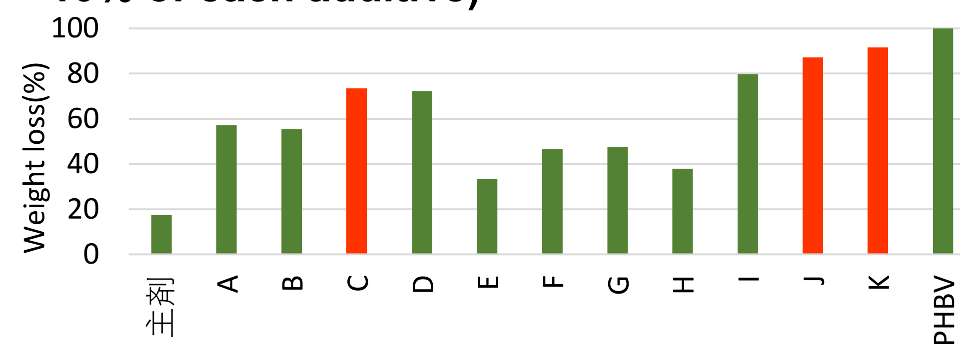
Candidates

Soybean-derived mass-produced products,

Rice bran, ...

Addition of soybean-derived mass-produced products (C, J, K) gave it good biodegradability
(Target value: 90%)

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



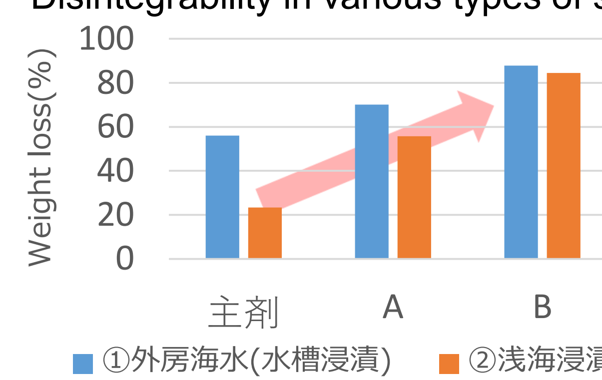
● Establishment of technology for dispersion of degradation accelerator(L compound)s in biodegradable resins and molding technology

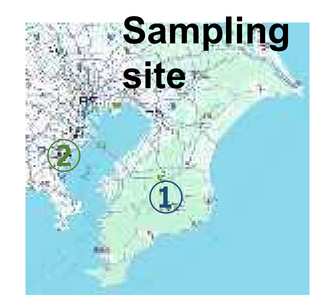
- **Film molding by inflation method (assuming packaging films, etc.)**
- **Improvement of dispersion by optimization of composition and blending of the third component**

Combination of decomposition and initial mechanical properties*.

＊： JIS Z1702

Disintegrability in various types of seawater (4 months)





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

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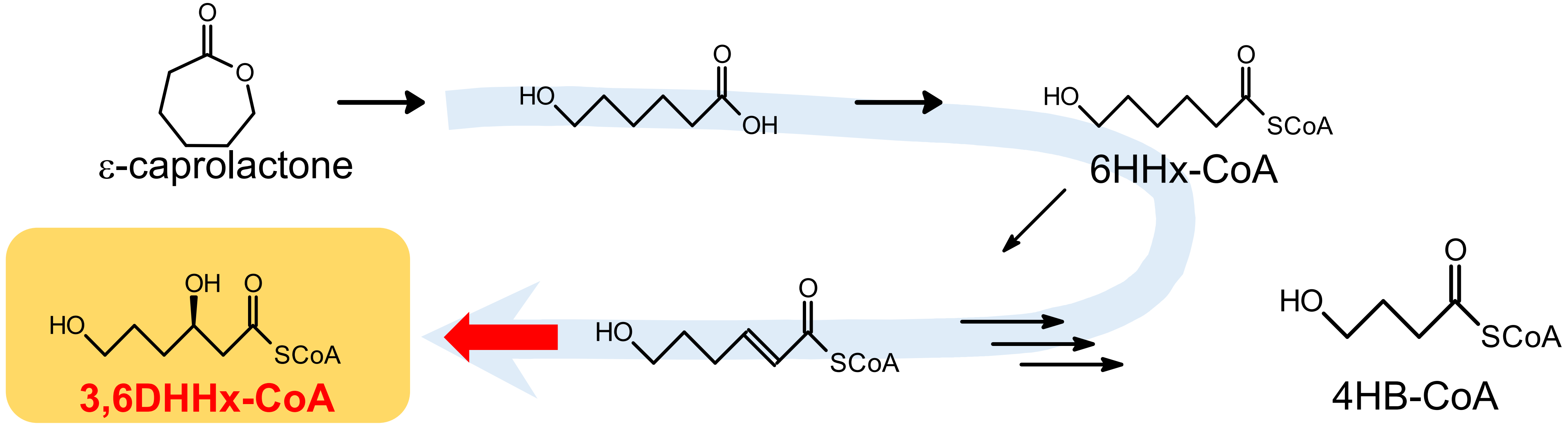
SI

R & D for social implementation

kaneka

Synthesis of PHA for installing switching functions

Biosynthesis of PHA with hydroxyl groups on the side chain



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

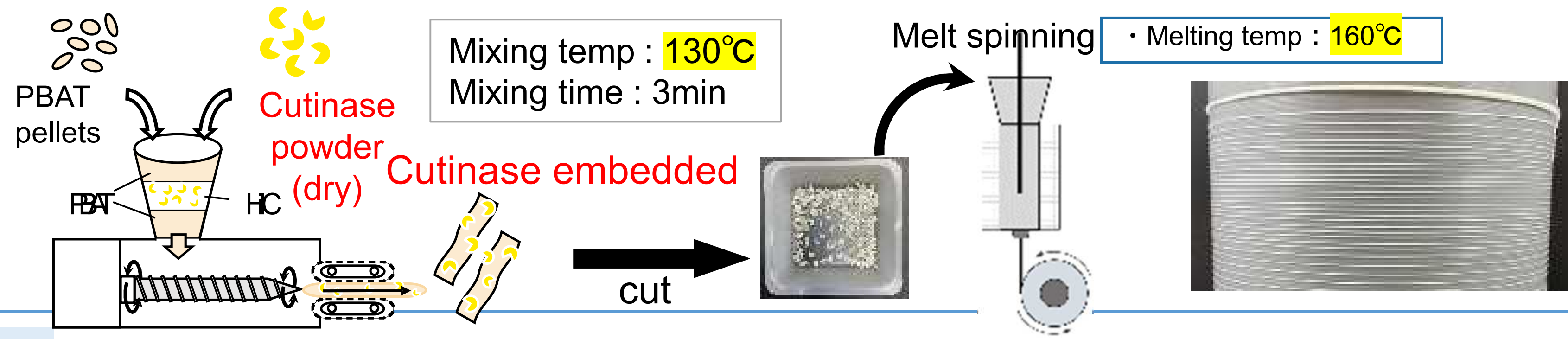
Host: *C. necator* mutant Carbon source: 15 g/L Fructose, 2.5 g/L ε-caprolactone

SI

R & D for social implementation

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.



● 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.

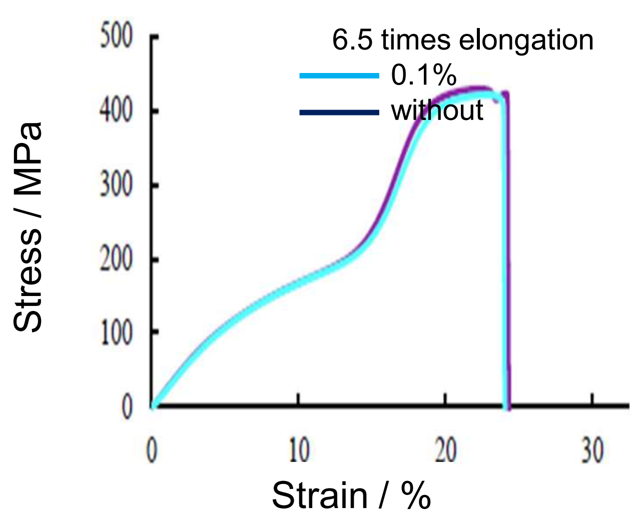
●Development of enzyme production technology

The investigation of mass-producing enzymes with both thermostability and degrading activity is underway.

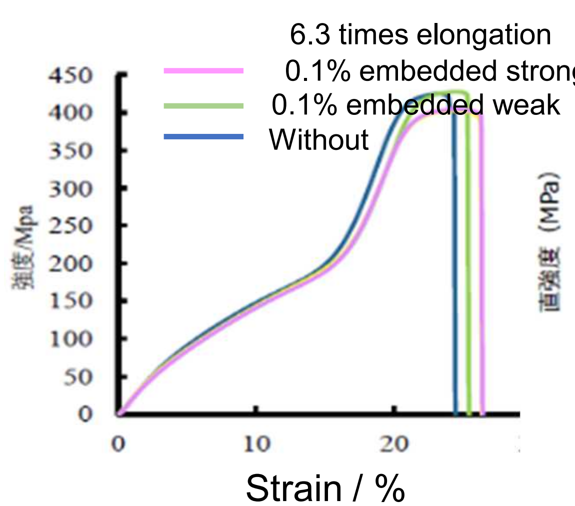
● Development of enzyme addition technology

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

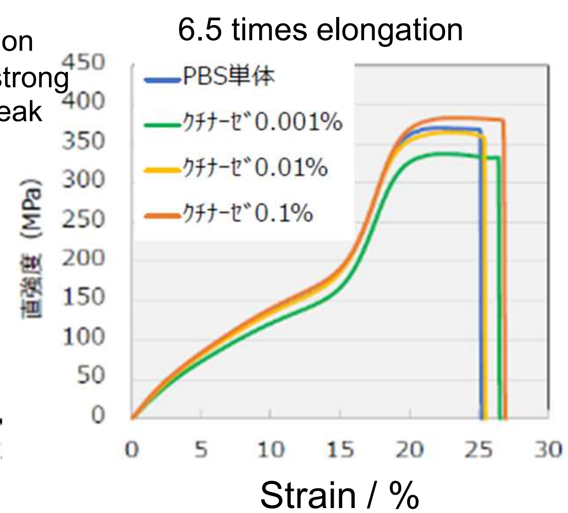
（1） Lipase

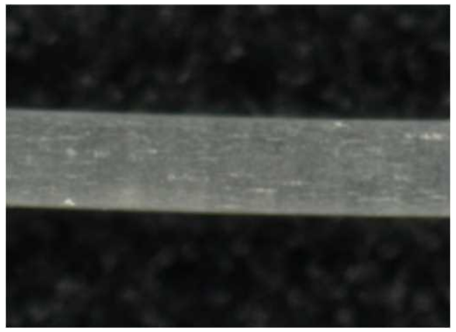


（2） Albumin



（3） Cutinase





PBS fiber with 0.1% cutinase

Marine biodegradability test will be conducted.

SI R & D for social implementation

Promote social implementation of marine biodegradable plastics based on the technology developed in PJ in collaboration with various companies

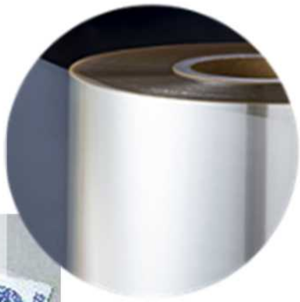
レンゴー

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.

JAMSTEC

国立大学法人 群馬大学 GUNMA UNIVERSITY



日本カーリット株式会社 Japan Carlit Co.,Ltd.

国立大学法人 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.・・・**

To create a clean earth for the future

Thank you for your attention.

MOONSHOT

RESEARCH & DEVELOPMENT PROGRAM