No. A-11-14E

PJ: Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses

Theme: Development of polymer from non-edible biomass（E3-1）

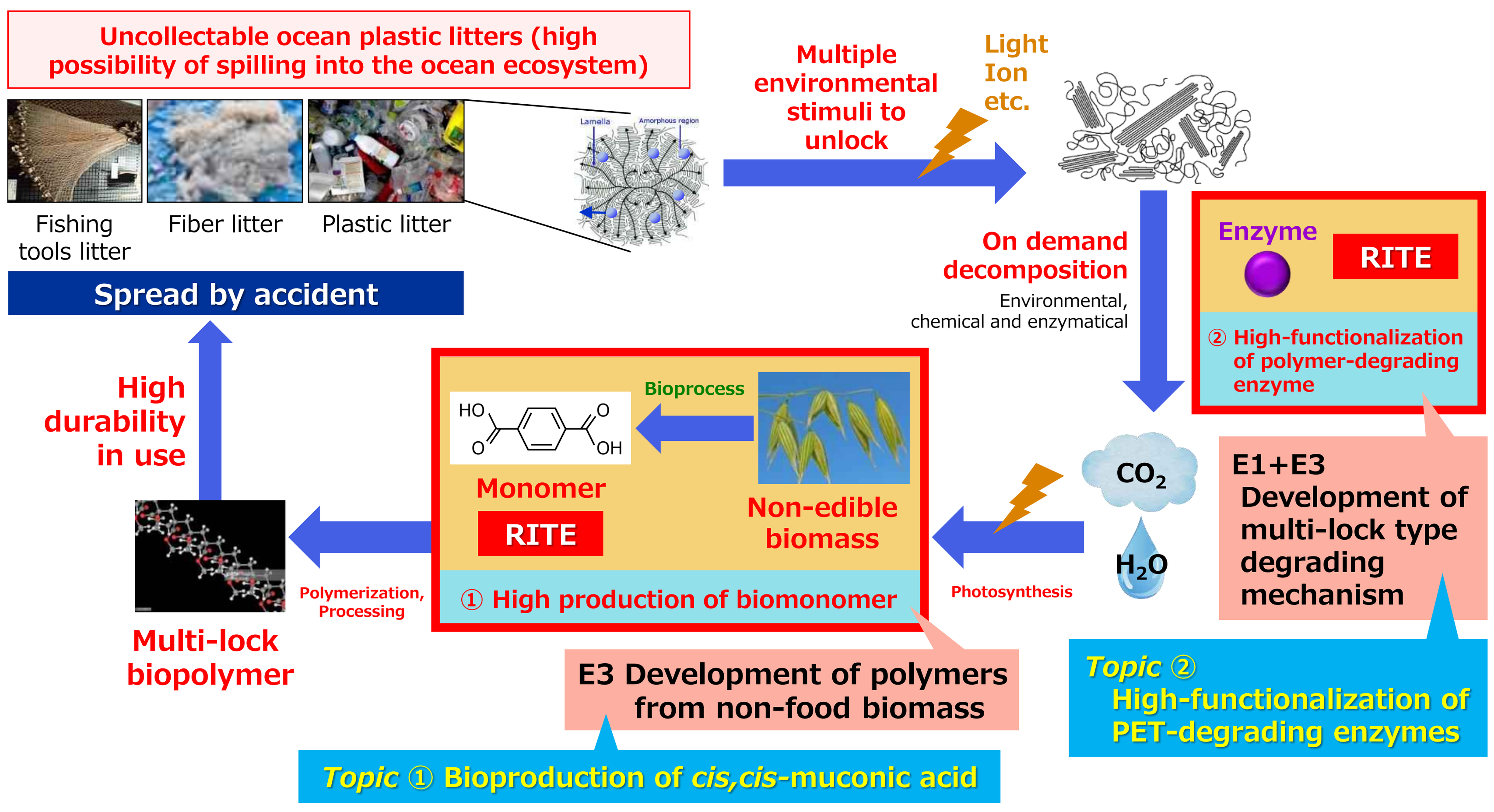
Organization: Research Institute of Innovative Technology for the Earth（RITE）

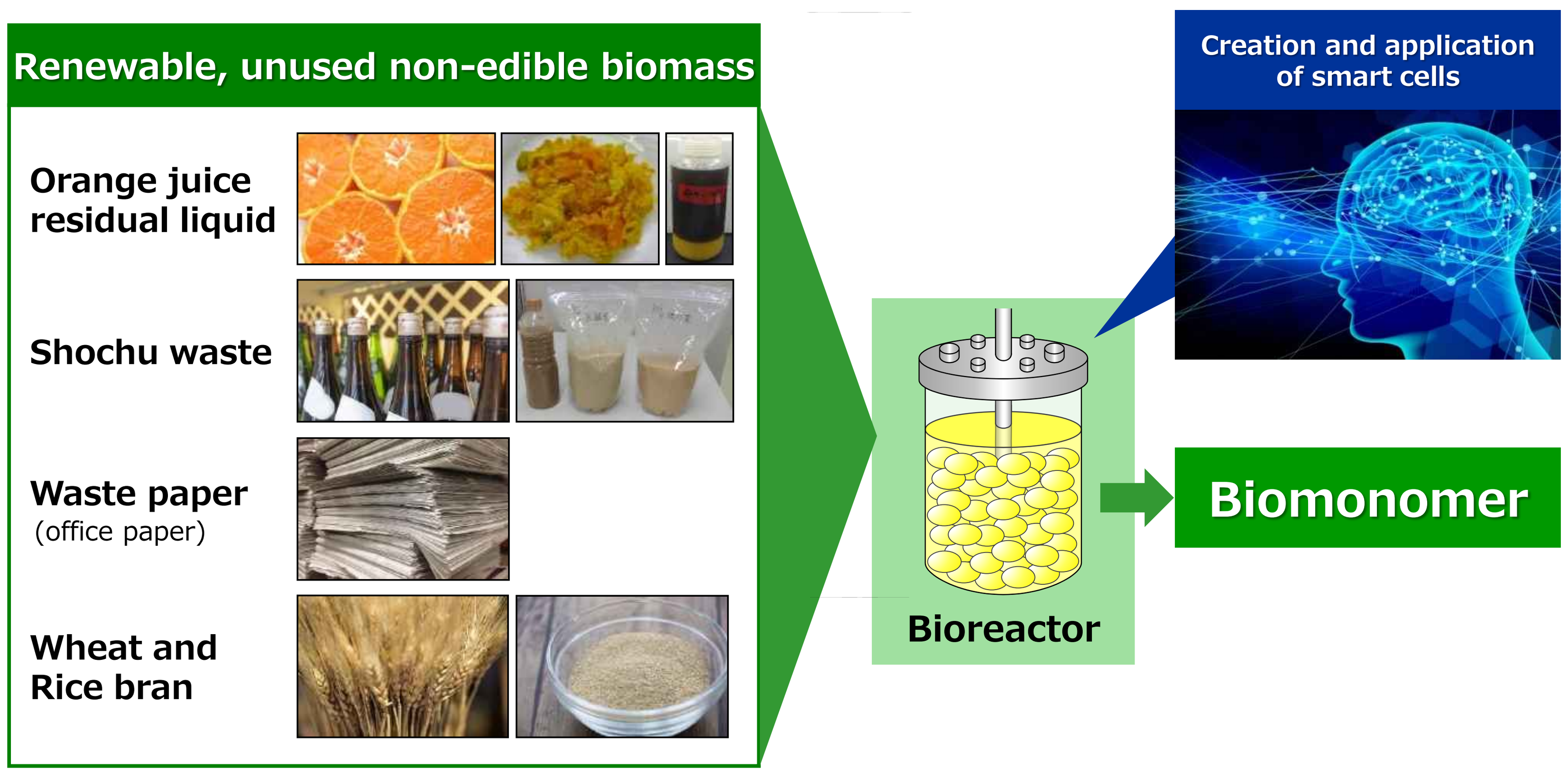
Contact: Masayuki Inui, inui@rite.or.jp（T. Shimizu, J. Kobayashi, M. Suda, Y. Tanaka, K. Hiraga）

【R&D items of RITE】

① High production of biomonomer

② High-functionalization of polymer-degrading enzymes



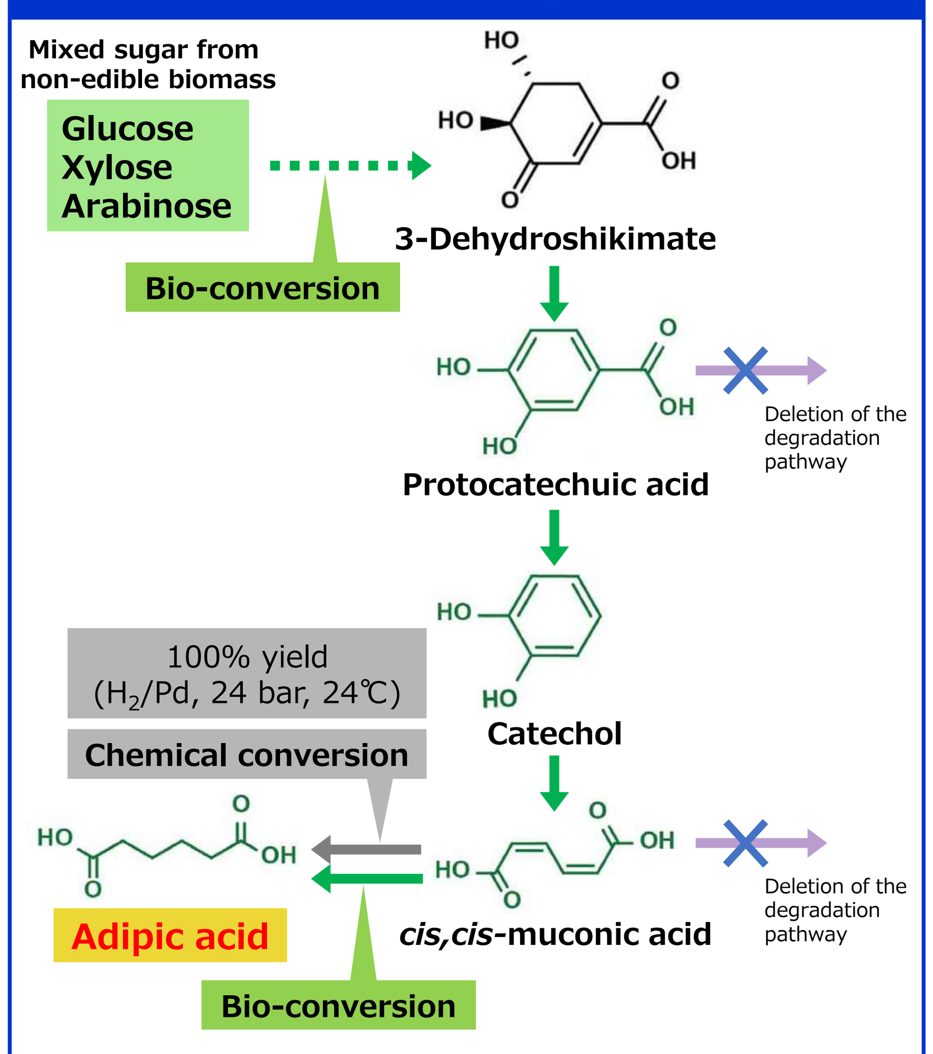


Utilization of non-edible biomass (including industrial waste)
= Contribution to carbon neutral
→ Great social and economic impact
(contributes to MS's Cool Earth)

Bioproduction of *cis,cis*-muconic acid

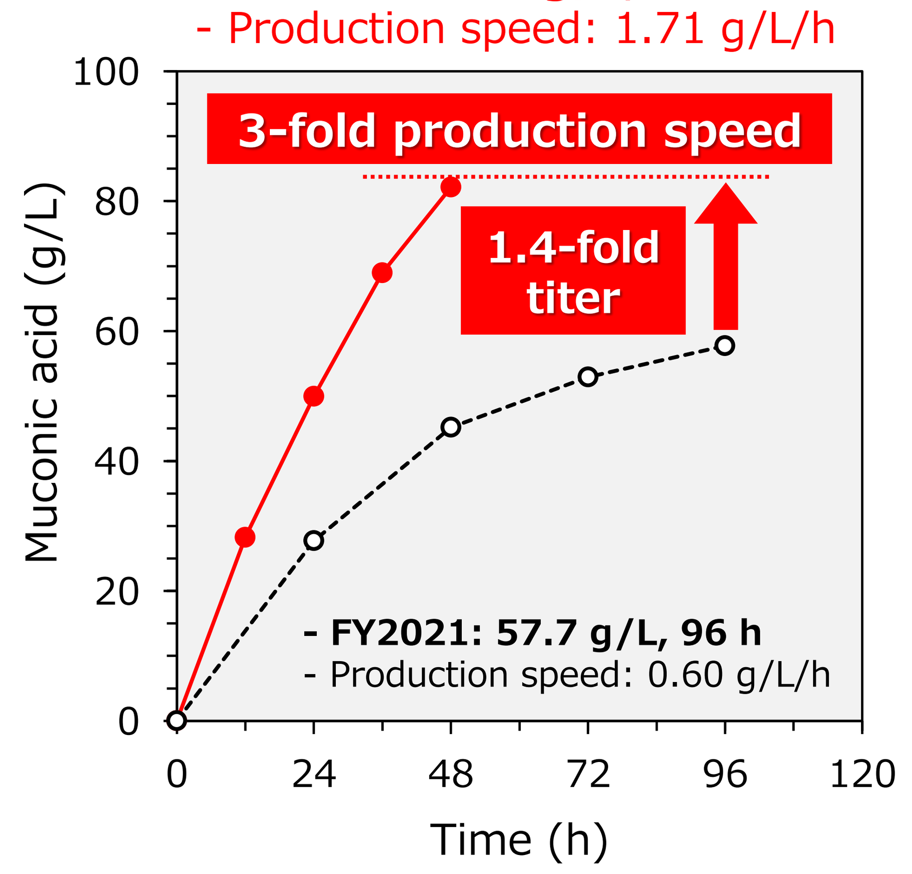
- Successful bioproduction of *cis,cis*-muconic acid, precursors of adipic acid, a raw material monomer for polyamides and polyesters (fishing tools, fibers, bottles, etc.).
- Screening of various genes for adipic acid-producing enzymes is in progress.

Artificial pathway for *cis,cis*-muconate production

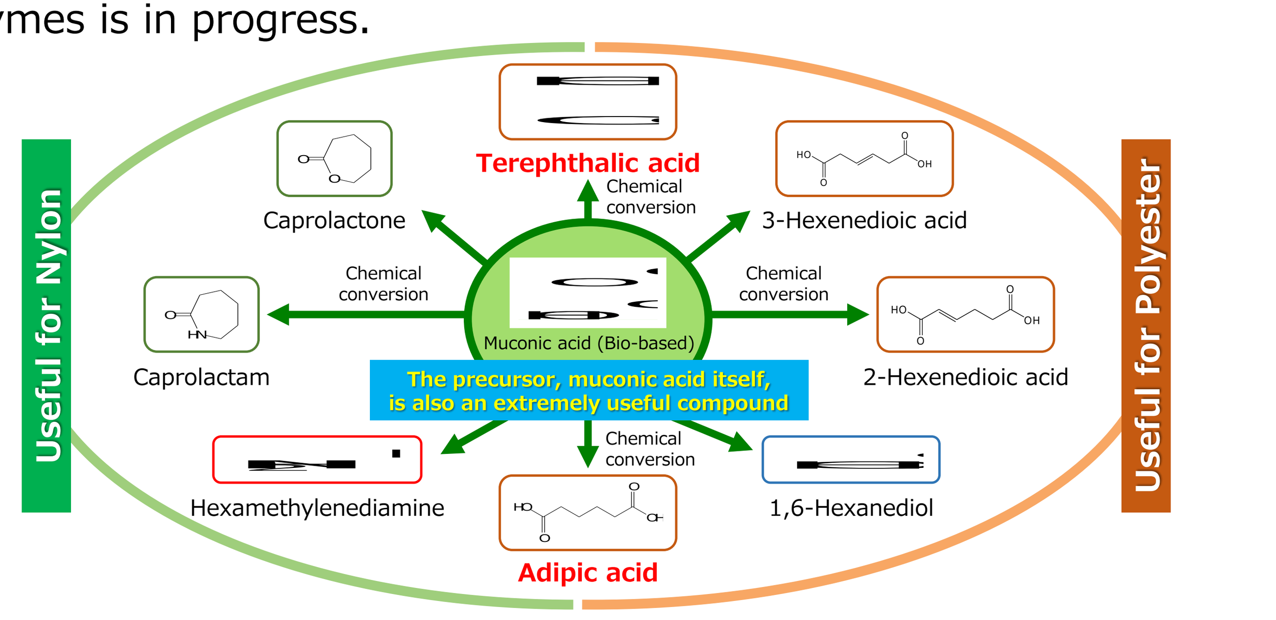


Time course of *cis,cis*-muconic acid production

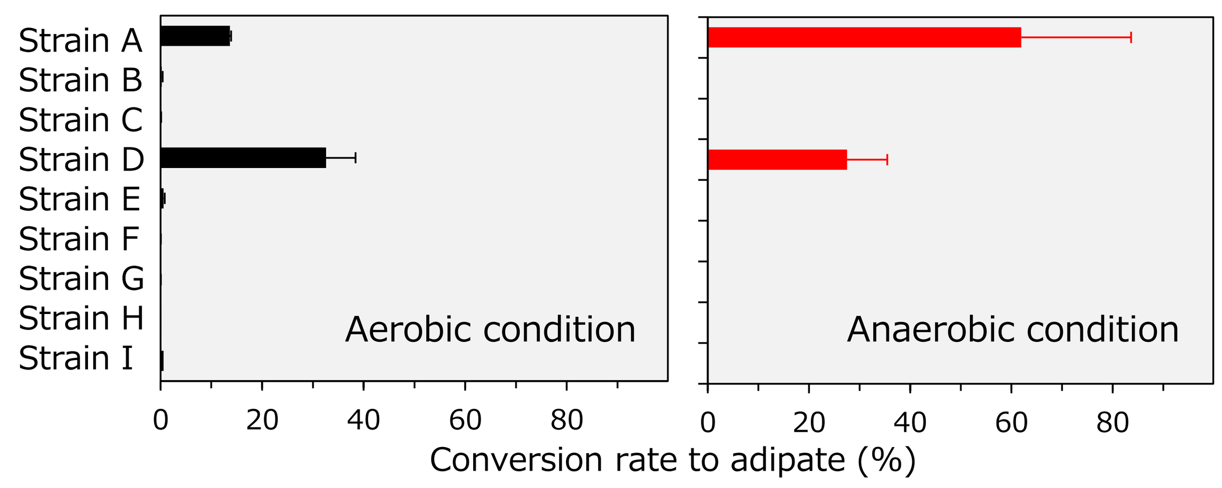
- FY2022: 82.1 g/L, 48 h
- Production speed: 1.71 g/L/h



250 mL Jar (fed-batch)
Existed as a sodium muconate in broth



Screening of adipate-forming enzymes (found from 44 candidates)

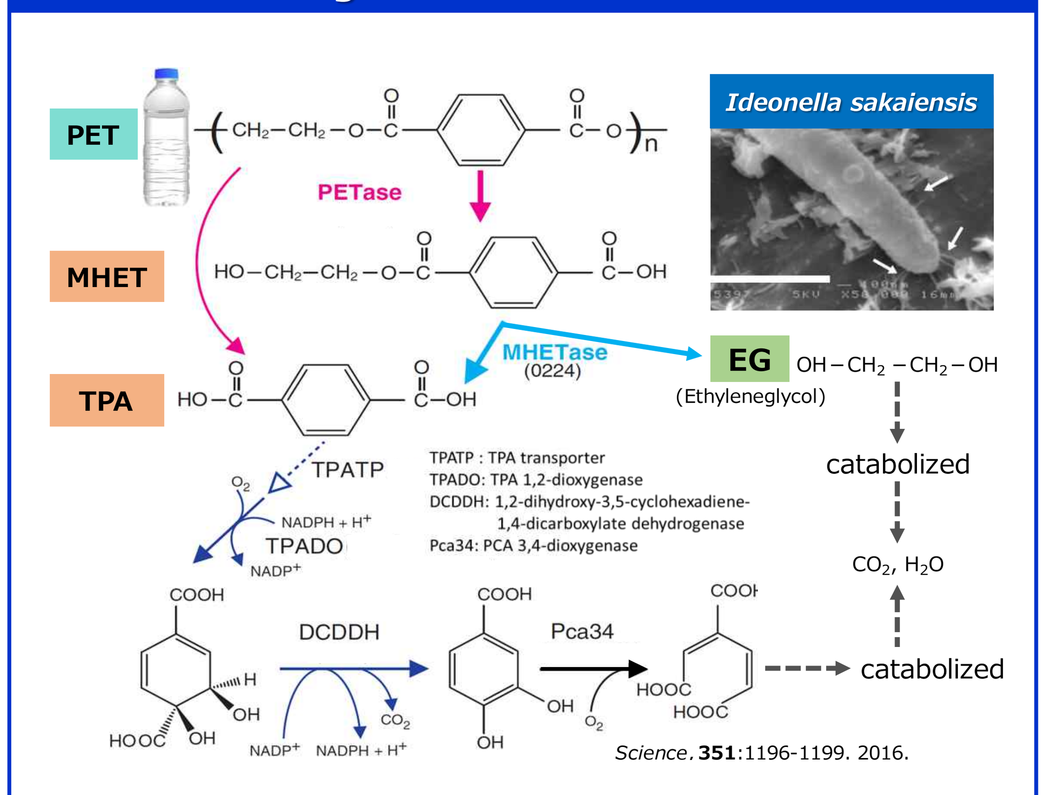


Result of enzyme activity: 8.5 mM *cis,cis*-miconic acid, 33℃, 200 rpm, 48 h

High-functionalization of PET-degrading enzymes

- Successful high-functionalization and high-production of a PET-degrading enzyme.
- Identification of degradation products of a PET-oligomer by a PET-degrading enzyme.

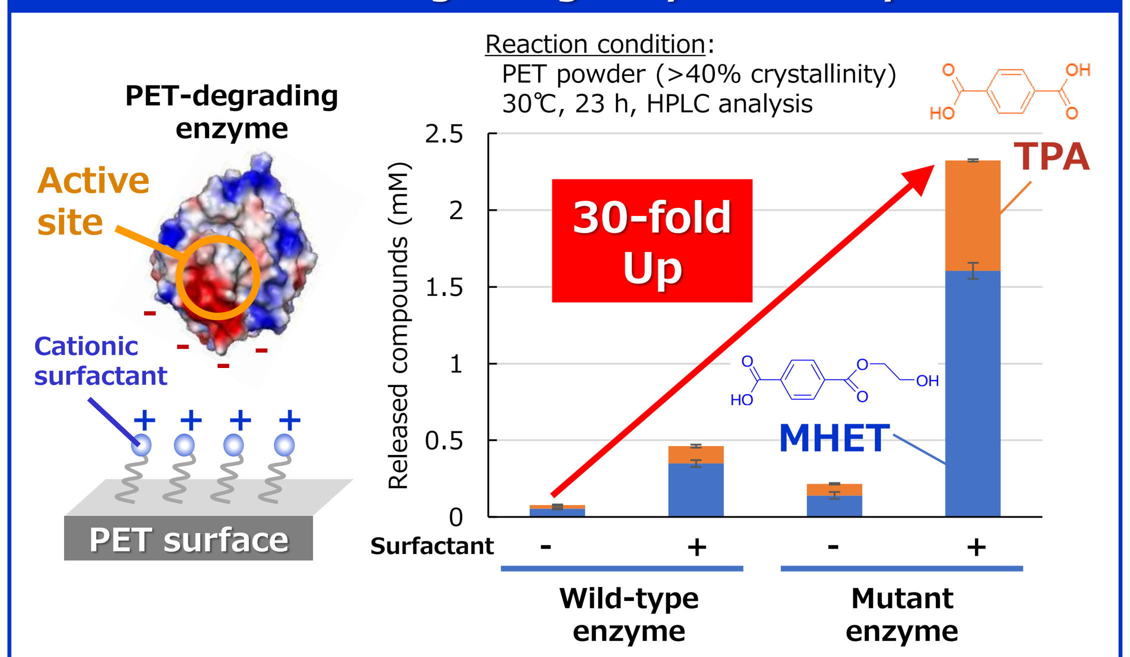
PET-degrading bacterium and degradation mechanism



Science. **351**:1196-1199. 2016.

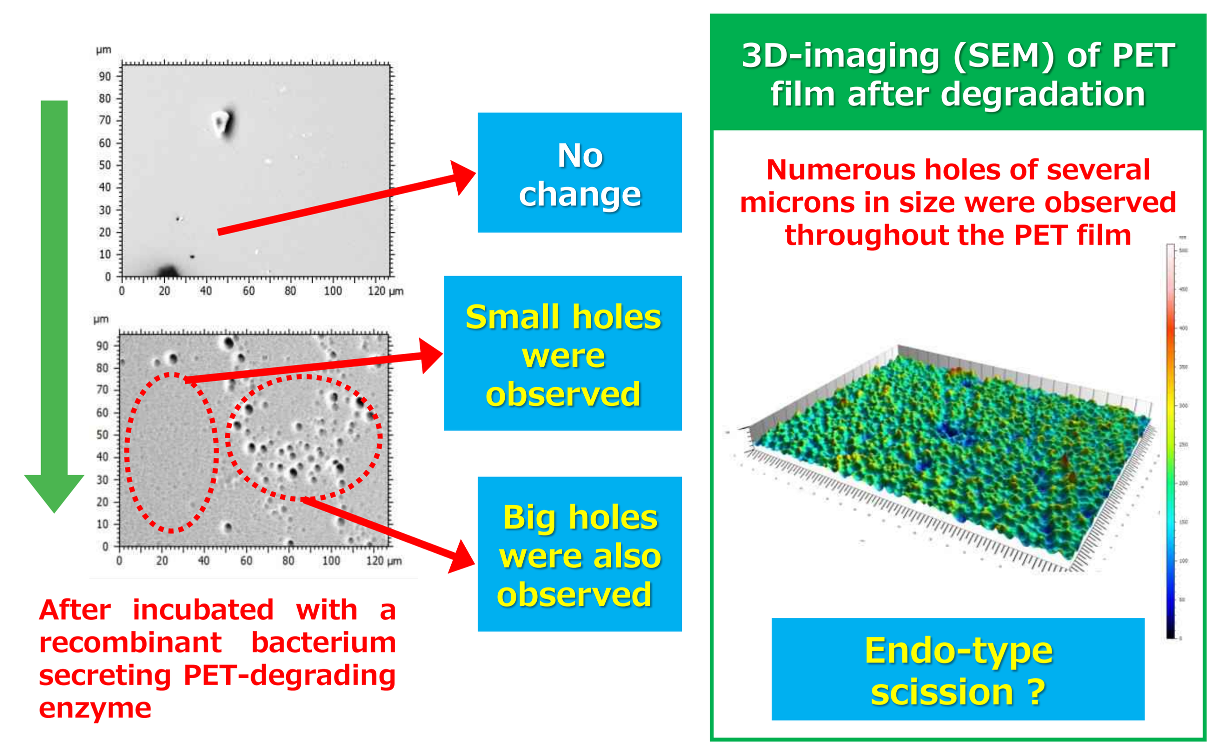
Effect of mutation and cationic surfactant on PET-degrading enzyme activity

Reaction condition:
PET powder (>40% crystallinity)
30℃, 23 h, HPLC analysis



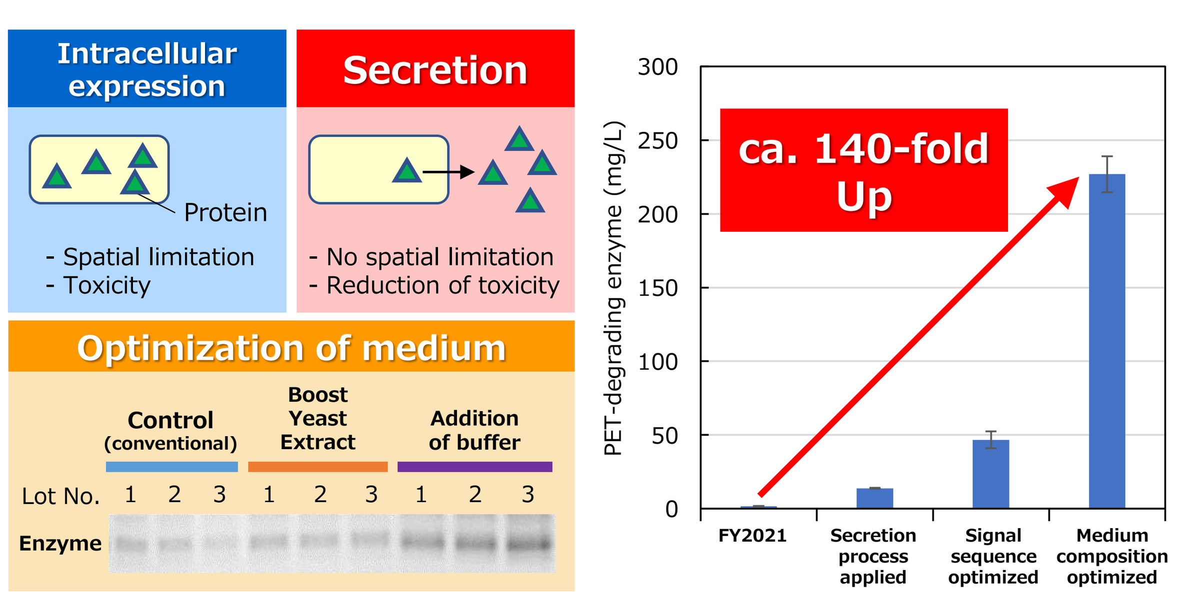
Addition of a very low concentration of cationic surfactant remarkably enhanced the enzyme activity about 30-fold comparing to the wild-type enzyme

Effect of mutation and cationic surfactant on PET-degrading enzyme activity

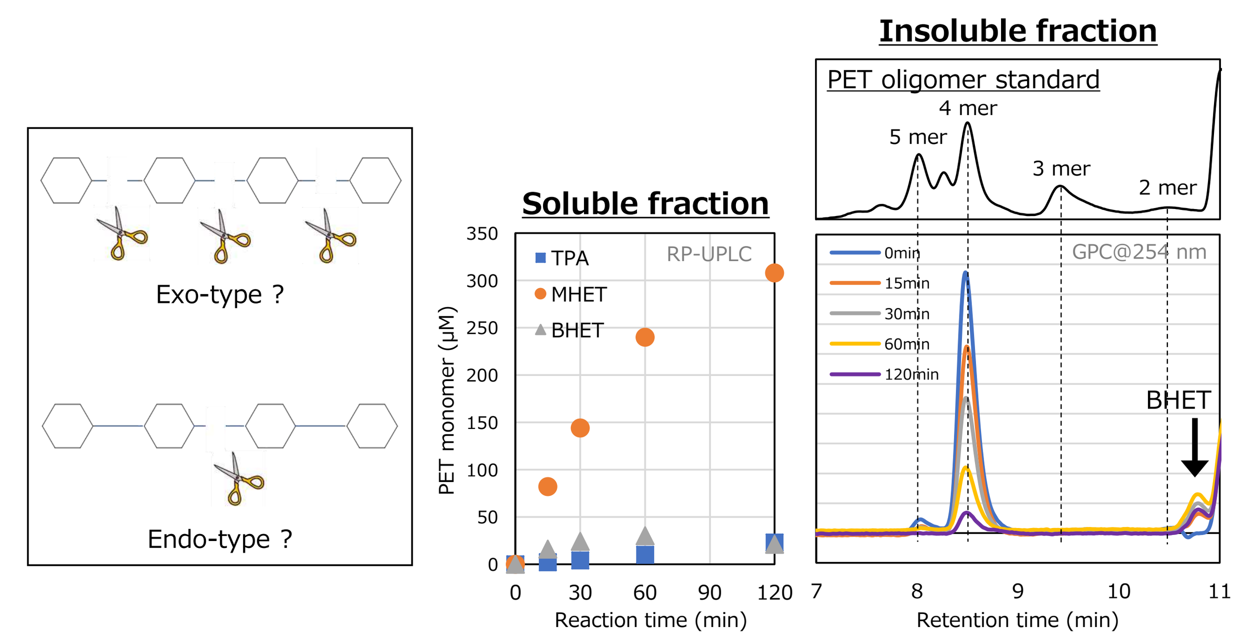


After incubated with a recombinant bacterium secreting PET-degrading enzyme

Overexpression of PET-degrading enzyme



Degradation products of PET-oligomer by PET-degrading enzyme



■ **Achievement of FY2022 targets**
- \>25 g/L monomer production.
- \>5-fold enzyme activity than WT-enzyme.

■ **Key points for future development**
- Select additional compounds in collaboration with chemical synthesis team.
- Development of switching function (regulation of degradation point and speed).
- Application of the biomonomer and PET-degrading enzyme to a multilock polymer.

No. A-11-15E

PJ: Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses

Theme: Control of Higher-Order Structure and Toughness of Marine Degradable Polymers through Polymer Processing (E3-2)

Organization: Yamagata University 山形大学 Yamagata University
Contact: Prof. Hiroshi Ito ihiroshi@yz.yamagata-u.ac.jp

1. Achievement in the PJ

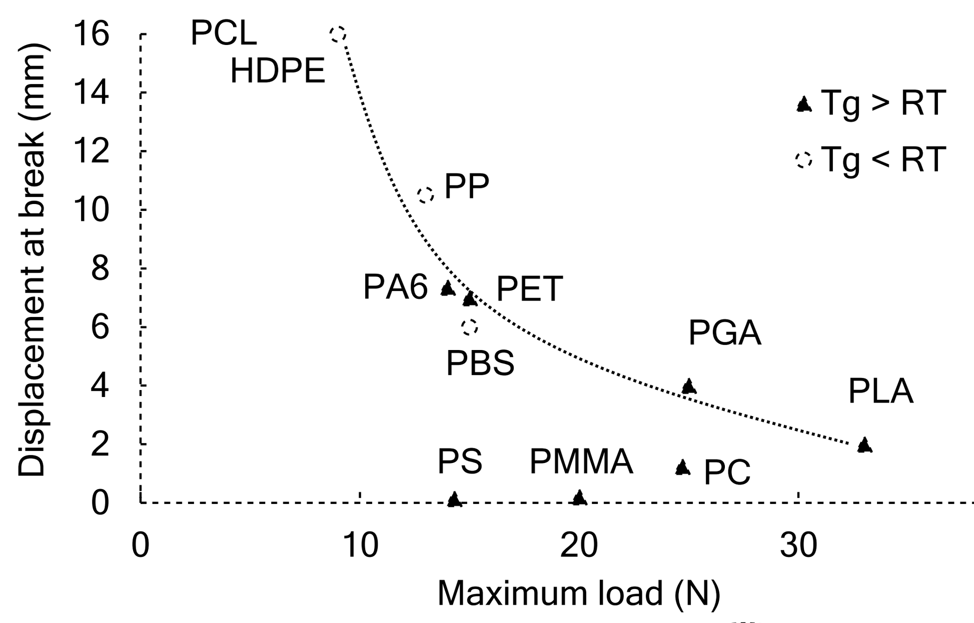
(1) Academic target

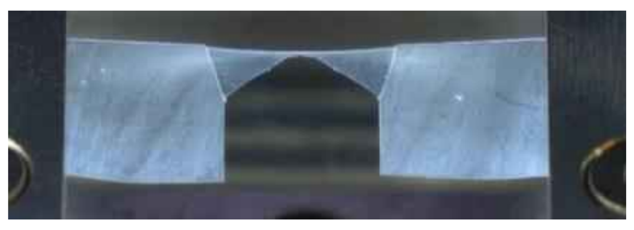
Improving durability and toughness of marine-degradable polymers

(Control crystal morphology for the marine degradation)

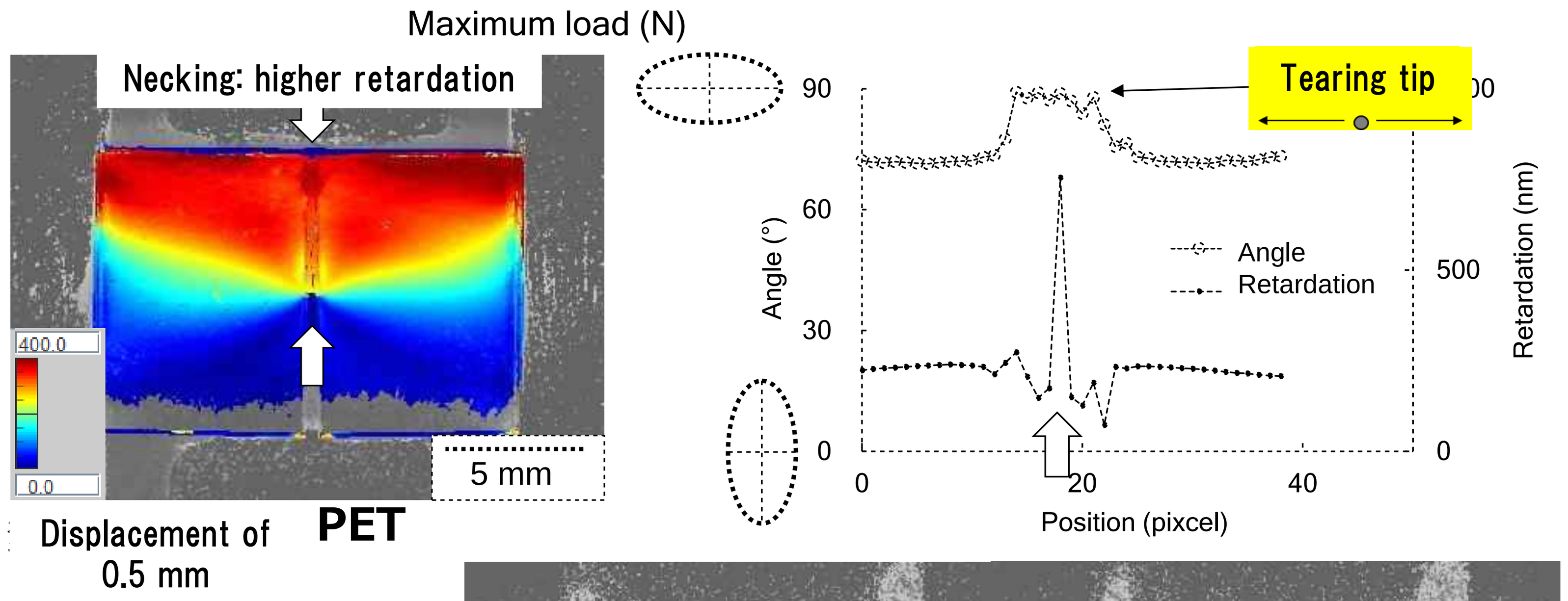
Propose toughening method through mechanical tests

	PLA	PGA	PMMA	PA6	PS	PC	PET	PBS	PCL	HDPE	PP
Tg (°C)	60	40	100	50	90	145	70	-35	-65	-110	0
Max. load (N)	33	25	20.3	14	14.3	24.7	15	15	8	9	13
Max. displacement (mm)	2	4	0.2	7.35	0.14	1.24	7	6	>16	16	10.5
Apparent modulus (MPa)	3510	4040	3370	890	3000	2660	2170	745	452	1030	1460



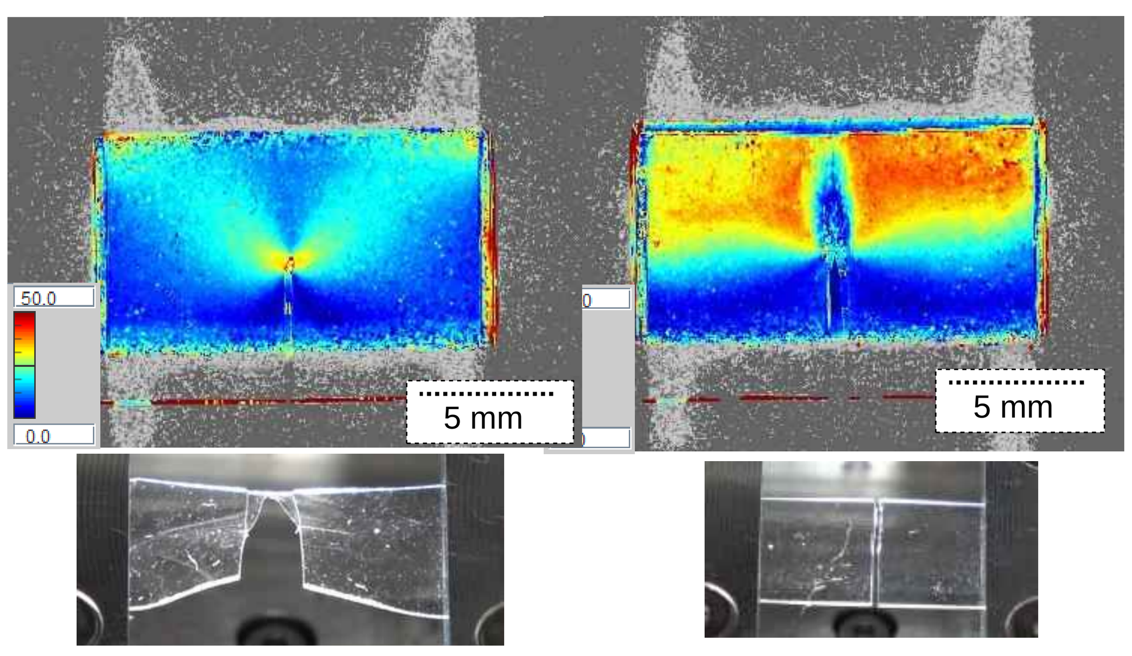


Photograph of tearing PET film just before break



PLA undergoes physical aging in an environment of 30 ℃ and 90 %RH and becomes embrittled while remaining amorphous.

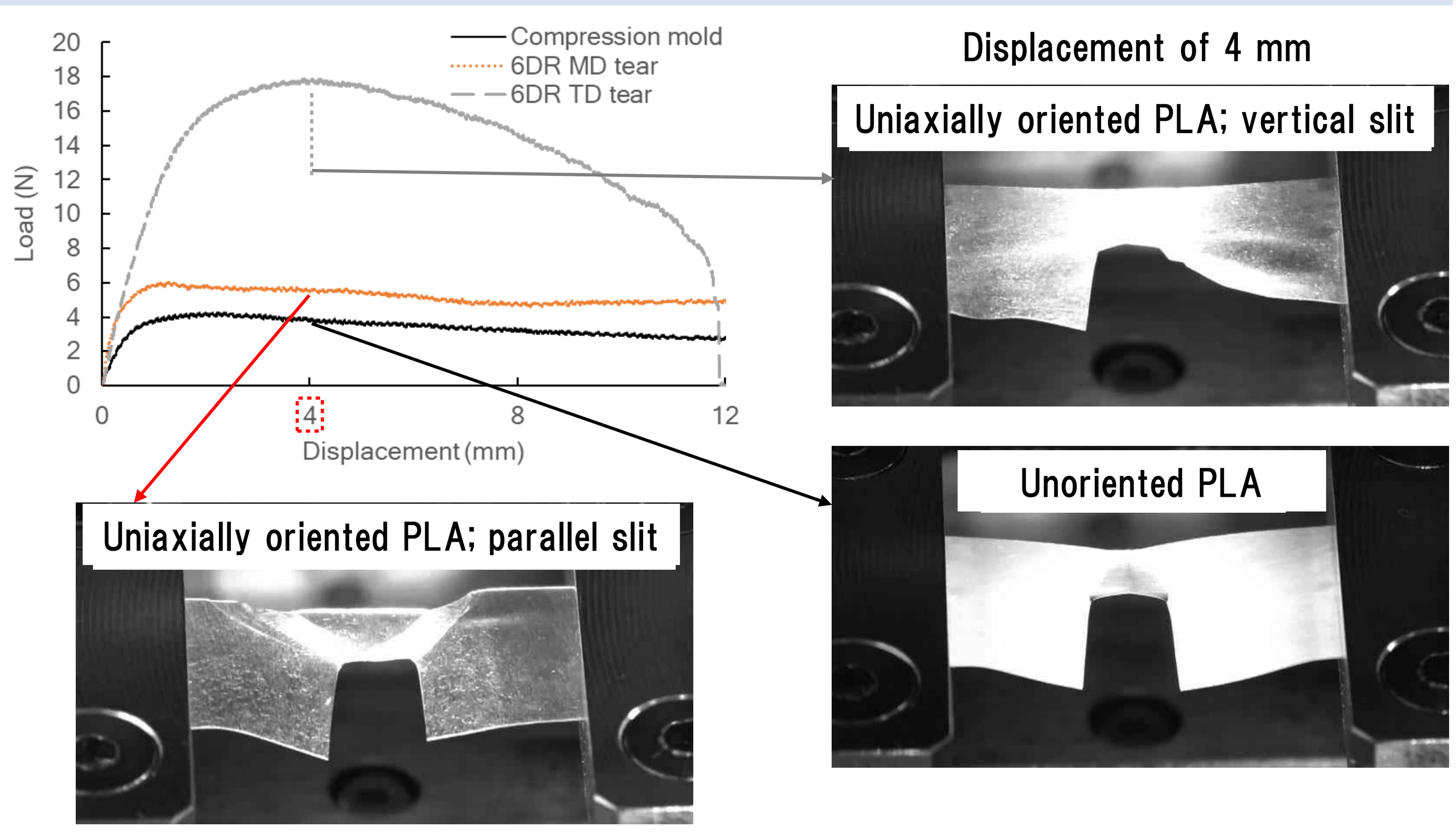
How to toughen?



PLA before physical aging

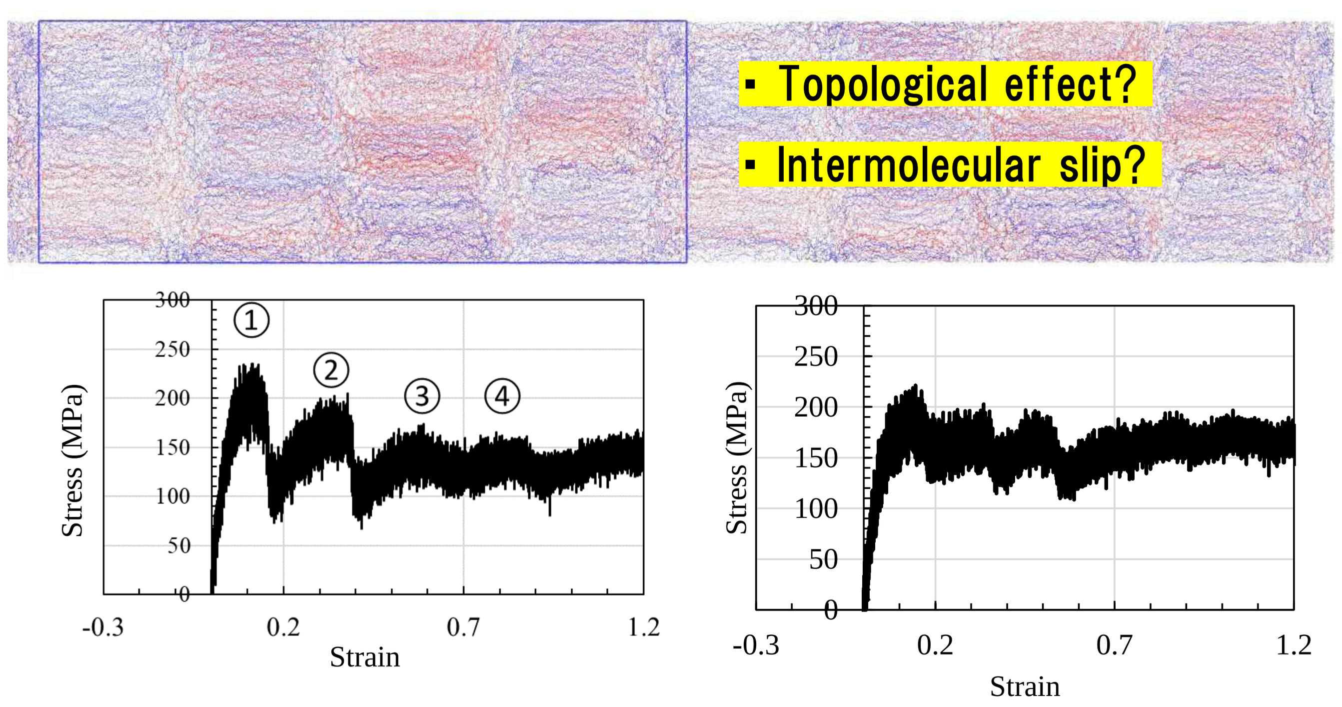
PLA after physical aging

Controlling crystal morphology with processing technologies



(3) Collaboration in Academia (With Prof. S. Okazaki, U.Tokyo)

Compare PCL to PBS with the same 4-layered lamella: PBS crystal is deformable



(2) Industry relations

Mitsubishi Chemical Corporation Project MITSUBISHI CHEMICAL GROUP

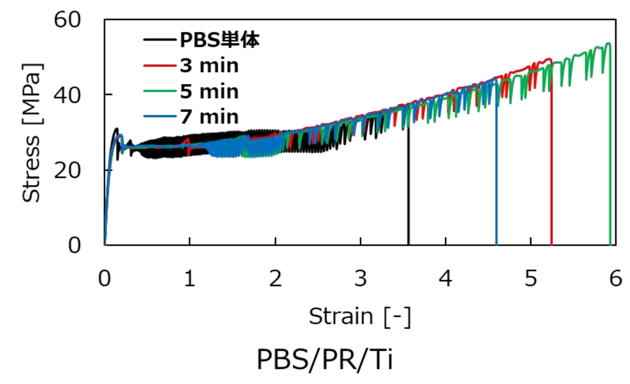
Research and development of marine degradable multi-lock biopolymers from inedible biomass

Toughening PBS films, especially improving tear strength using the polymer blend technique

Achieved the annual target of toughness by means of a fine dispersion of PR in PBS using an organotitanium catalyst.

PBS: BioPBS™ :FZ91PB
PR: SH3400P (ASM Inc.)
Organotitanium catalyst:
Tetra n-butyl titanate (TBT)
(Matsumoto fine chemical Co.)
ORGATIX® TA-21
$Ti(O-n-C_4H_9)_4$

Batch mixer
Labo Plastmill 4C150 15cc

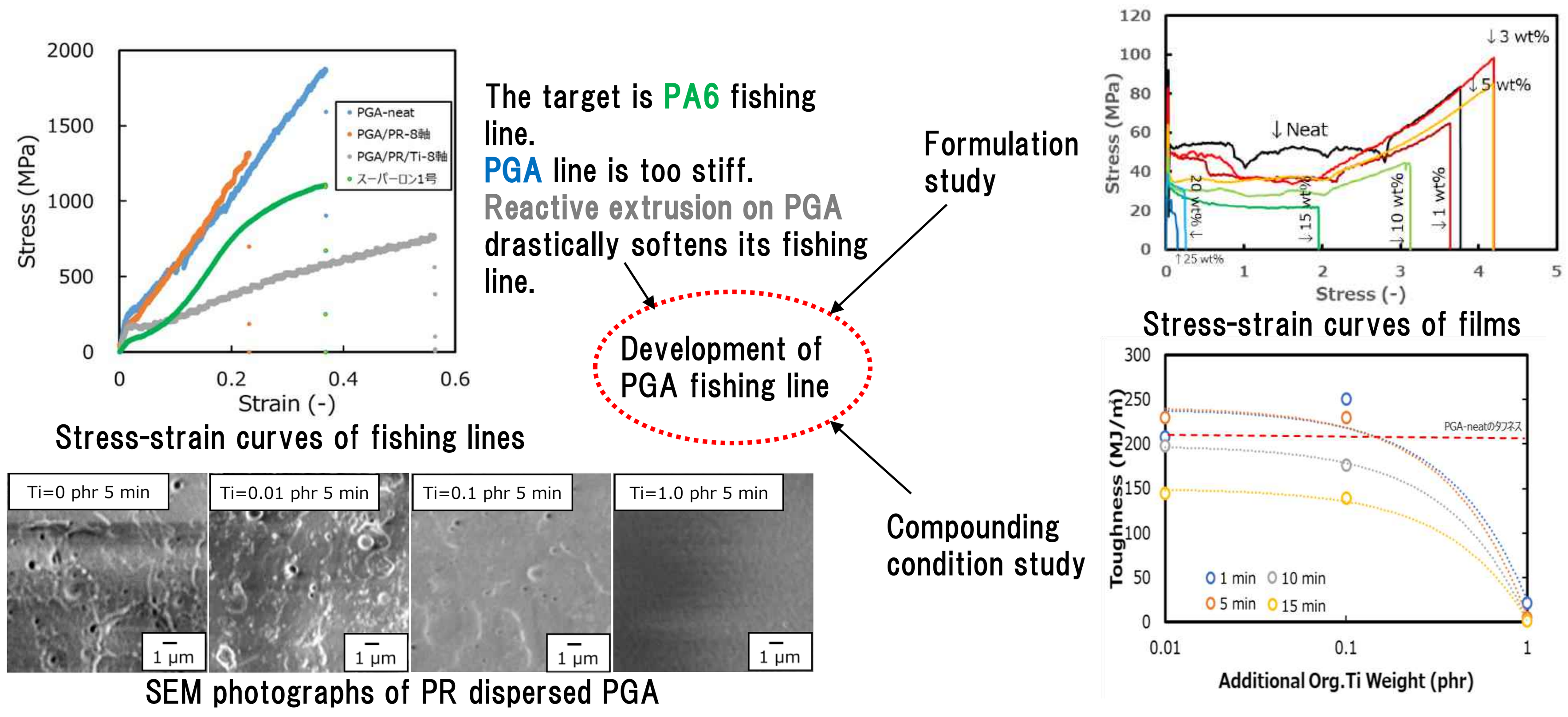


PBS/PR/Ti

KUREHA CORPORATION Project KUREHA

Development of strong and degradable biopolymers for fishing nets

Toughening PGA with special compounding.
Controlling PA4 crystal morphology using processing technique.

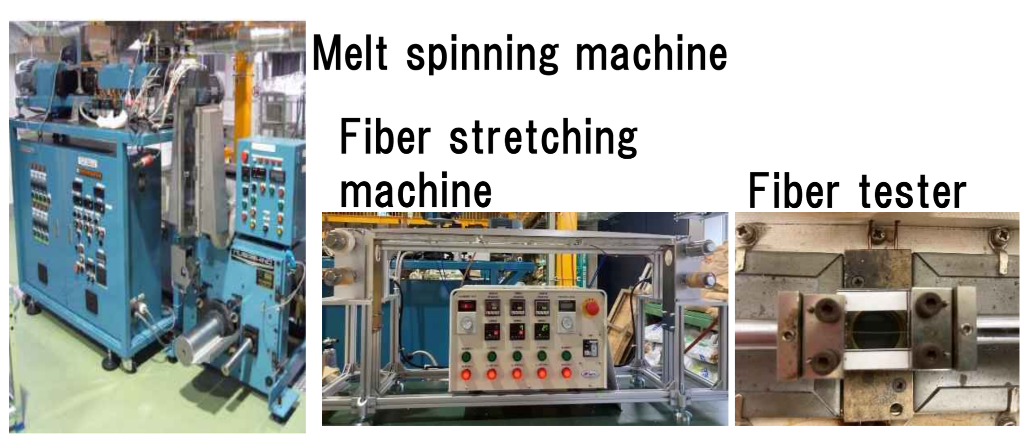


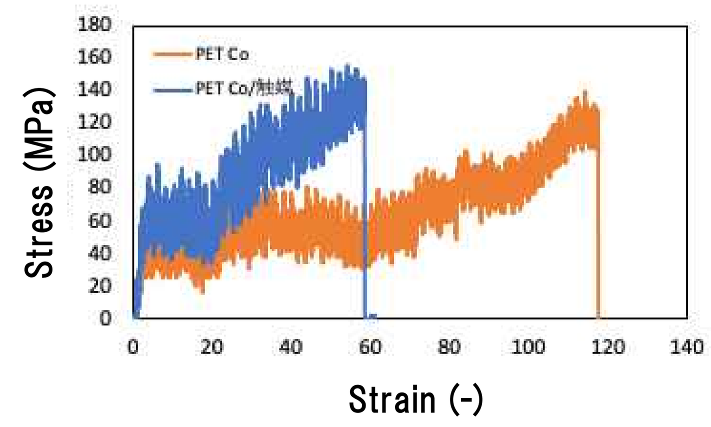
TEIJIN LIMITED Project TEIJIN

Development of highly degradable polyester-based multi-lock type bio-tough polymers and its fibers

Development of nano-structured PET and its melt spinning

Added a decomposition catalyst into PET. Doped PET showed brittleness on oriented filaments



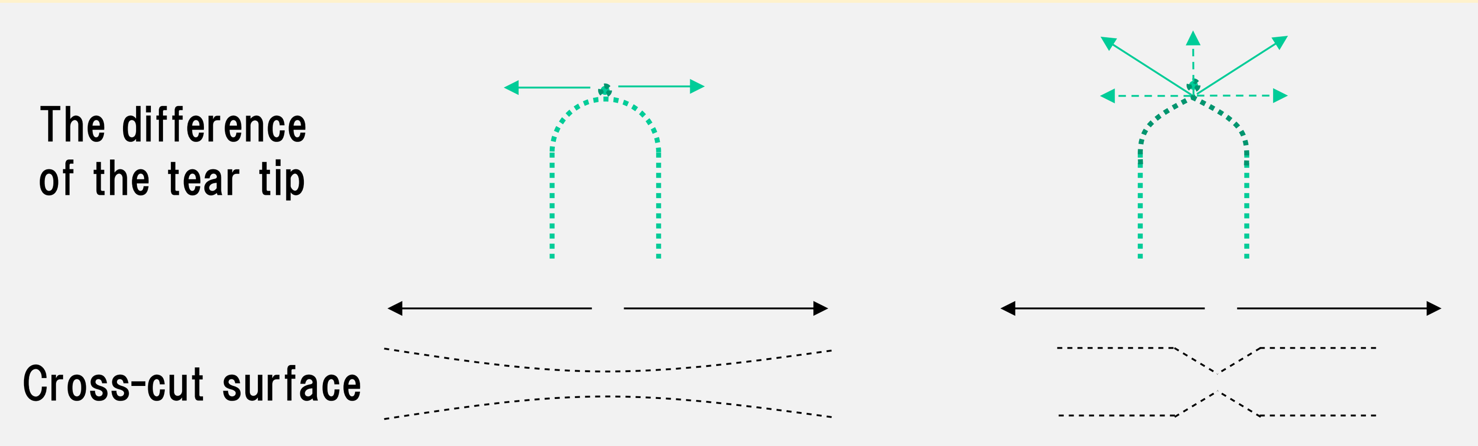


2. Next plan

(1) To realize marine-degradable plastics products with certain crystallinity
- Development of tough polymer blends and composites using special compounding technique

(2) To clarify principles between toughness and marine-degradability
- Toughening mechanism on tear strength
- Preparation of variety of crystal morphology and analysis of its degradability

Analyze the relationship between molecular structure and macroscopic tearing using the MD simulation ➡ Guideline for material development



No. A-11-16E

PJ: Development of Multi-lock Degradable Biopolymers in Ocean from Non-food Biomasses

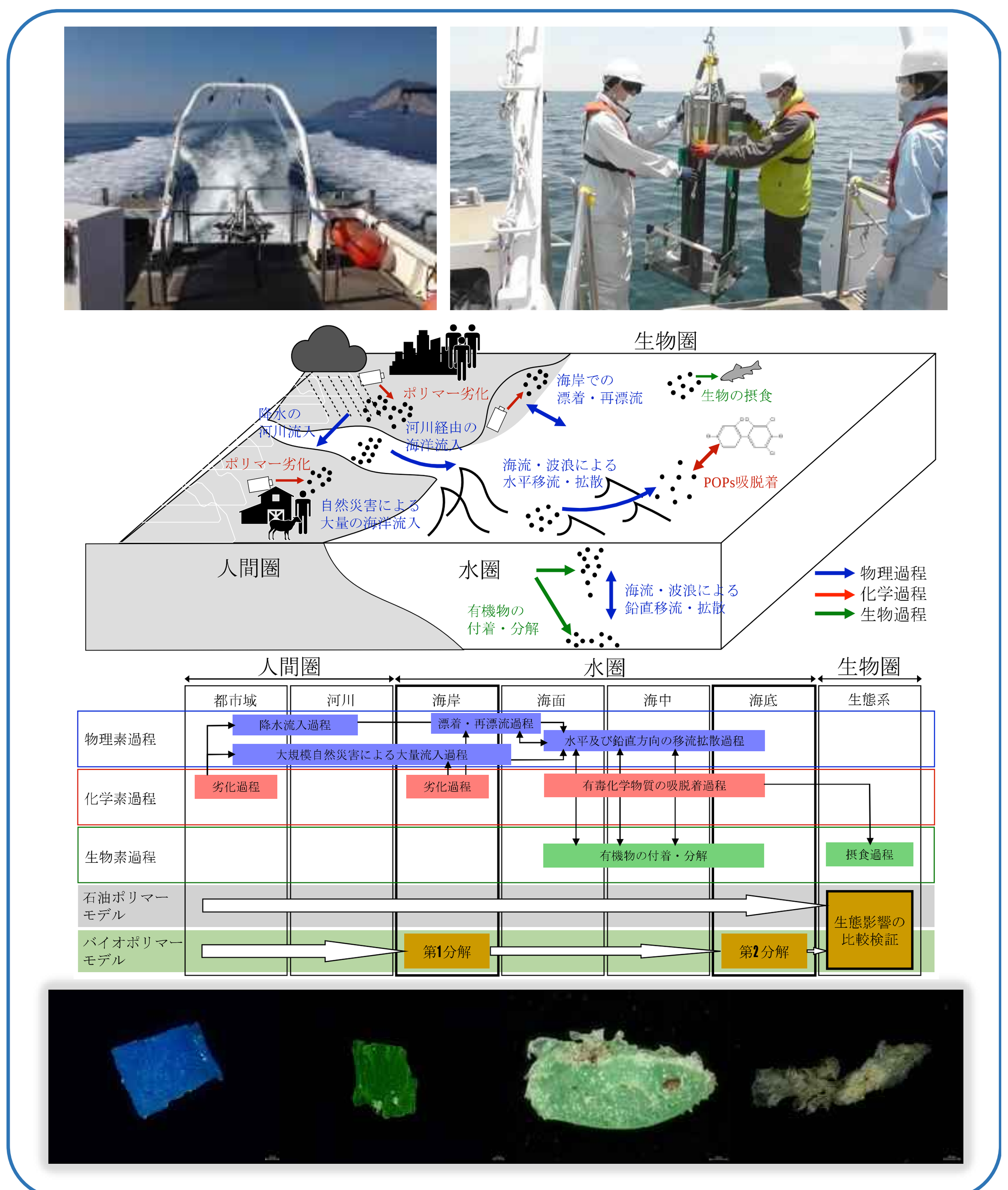
Theme: Development of a prediction model for long-term impacts of multilocked new polymers on the marine environment（E4）

Organization: Ehime University

Contact: Graduate School of Science and Engineering, Hirofumi Hinata（hinata.hirofumi.dv@ehime-u.ac.jp）

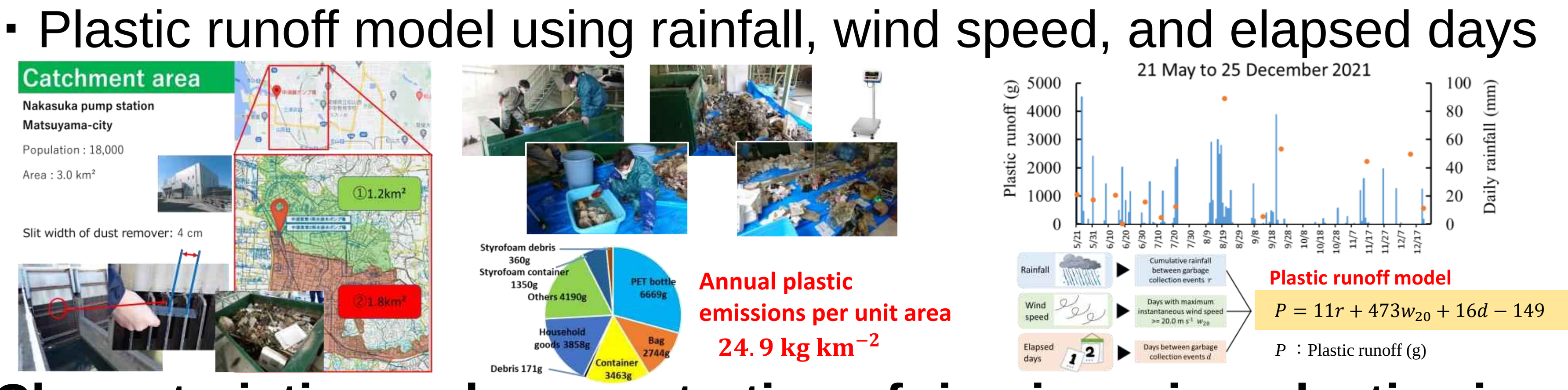
■Outline of our research

1) To develop a prediction model for the long-term impacts of new polymers on the marine environment consisting of physical, chemical, and biological models.
2) To understand the standing stocks in the marine reservoirs, such as water columns, beaches, bottom sediments, marine biota, and fluxes between them with the integrated model.
3) To comprehend the polymer behaviors in the marine environment and assess the impacts based on an input-output system approach.
Researches start with the Seto Inland Sea and then extends to the North Pacific.



■ Urban plastic runoff model

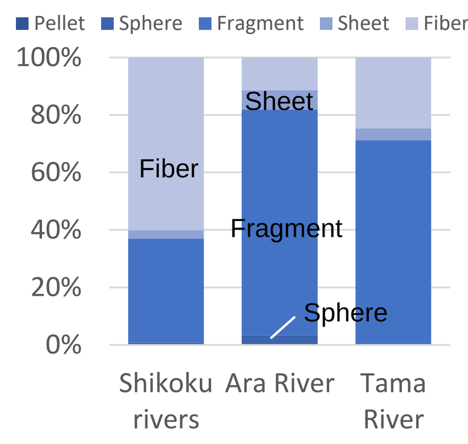
・Estimate of plastic unit runoff by weighing at pump station
・Plastic runoff model using rainfall, wind speed, and elapsed days



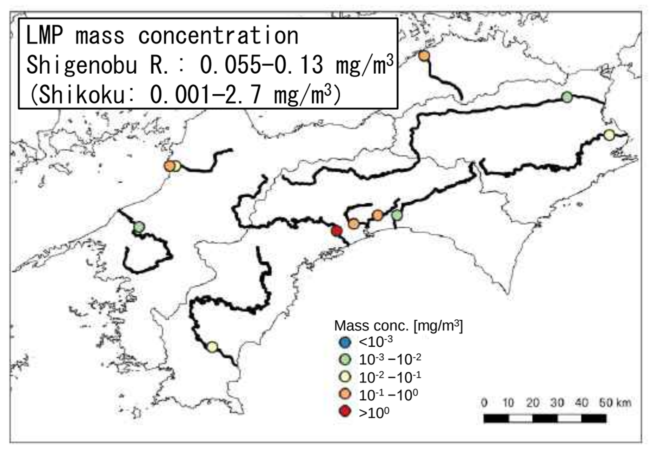
Characteristics and concentration of riverine microplastics in Shikoku region

・Fiber- and fragment-type LMP[*1] particles were predominantly distributed in rivers in Shikoku and Kanto regions, respectively.
・In Shigenobu River, SMP [*2] concentration/LMP concentration was between 10 to 100 times.

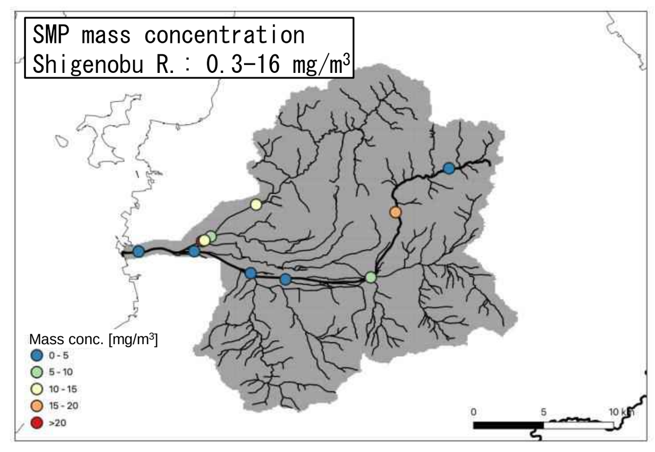
[*1]LMP:Large microplastics(≥0.3mm), [*2]SMP:Small microplastics(<0.3mm)



Composition of LMP in Shikoku and Kanto regions



LMP mass concentration in Shikoku rivers



SMP mass concentration in Shigenobu river basin

■ A model for the beach process and degradation process

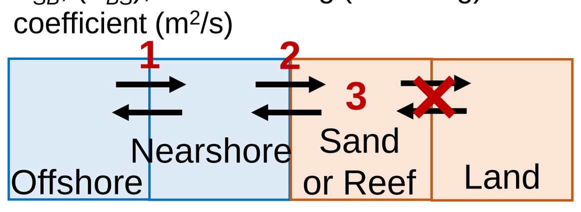
1. Governing Equation

$$\frac{\partial C}{\partial t} = -\left(\frac{\partial (uC)}{\partial x} + \frac{\partial (vC)}{\partial y}\right) + \frac{\partial}{\partial x}\left(K_h \frac{\partial C}{\partial x}\right) + \frac{\partial}{\partial y}\left(K_h \frac{\partial C}{\partial y}\right) + S$$

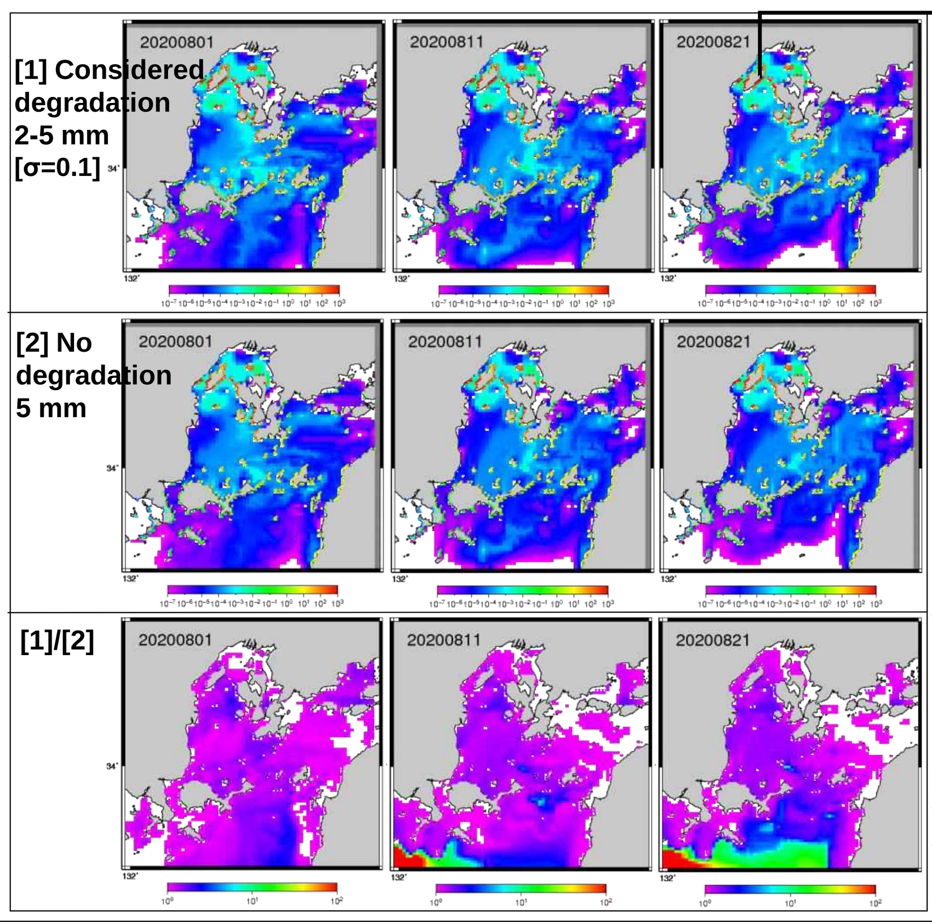
C; Concentration (-/m^2)
u,v; Current velocity (m/s)
K_h; Horizontal diffusion coefficient of sea (m^2/s)
S; Input flux (-/m^2・s)
K_{SB}, (K_{BS}); Backwashing (beaching) diffusion coefficient (m^2/s)

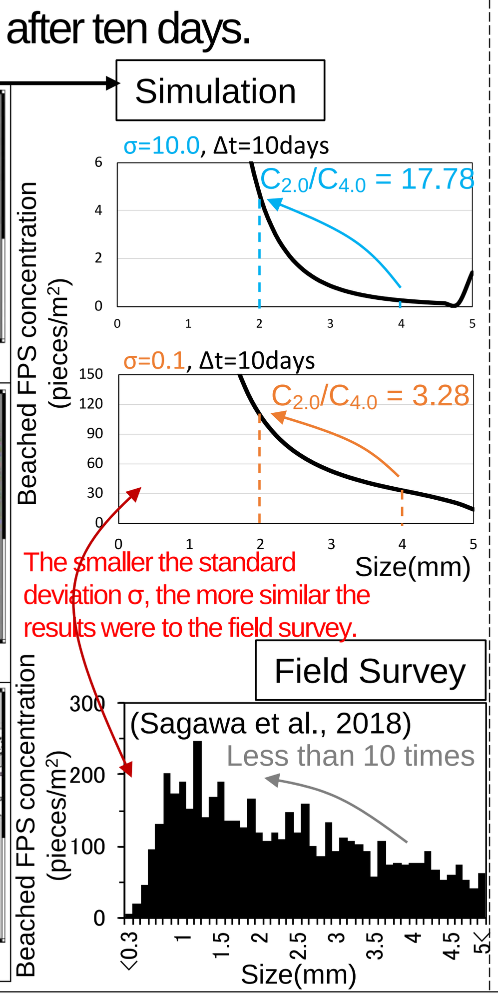
2. Beach process excluding reef grids

$$K_h \frac{\partial C}{\partial x} + K_h \frac{\partial C}{\partial y} = \left(K_{SB}\frac{\partial C}{\partial x} + K_{BS}\frac{\partial C}{\partial x}\right) + \left(K_{SB}\frac{\partial C}{\partial y} + K_{BS}\frac{\partial C}{\partial y}\right)$$



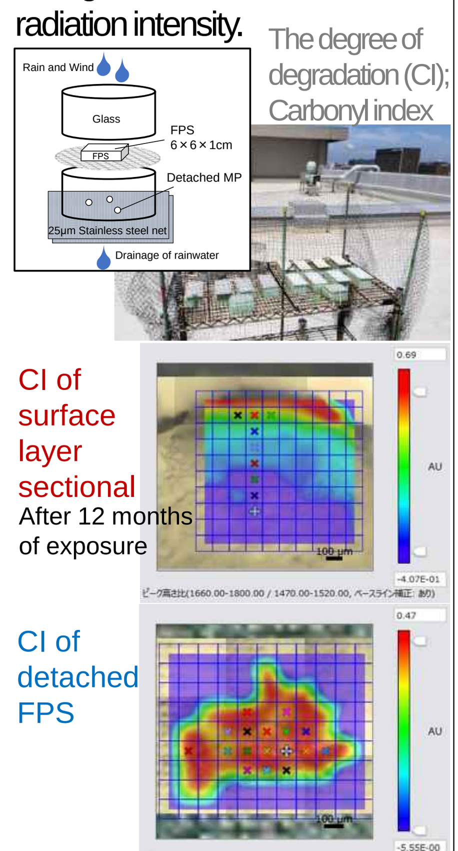
3. Degradation process; Size division based on the normal distribution curve was performed for each size of beached FPS after ten days.





Sunlight exposure

The model will reflect the amount of MP generated and the degree of degradation in relation to solar radiation intensity.



■ Transport of degradable biopolymers in Seto Inland Sea

➢ **Governing Equation:**

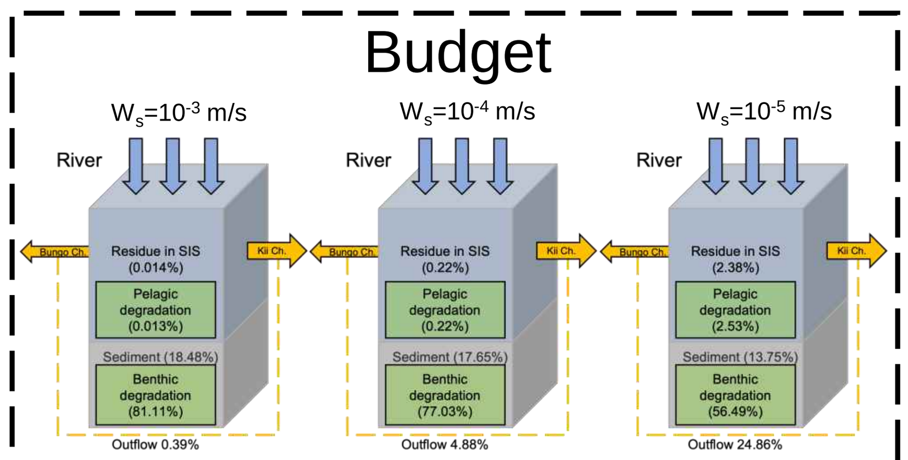
$$\frac{\partial C}{\partial t} + \frac{\partial UC}{\partial x} + \frac{\partial VC}{\partial y} + \frac{\partial (W - W_s)C}{\partial z} = \frac{\partial}{\partial x}\left(A_H \frac{\partial C}{\partial x}\right) + \frac{\partial}{\partial y}\left(A_H \frac{\partial C}{\partial y}\right) + \frac{\partial}{\partial z}\left(K_H \frac{\partial C}{\partial z}\right) + S$$

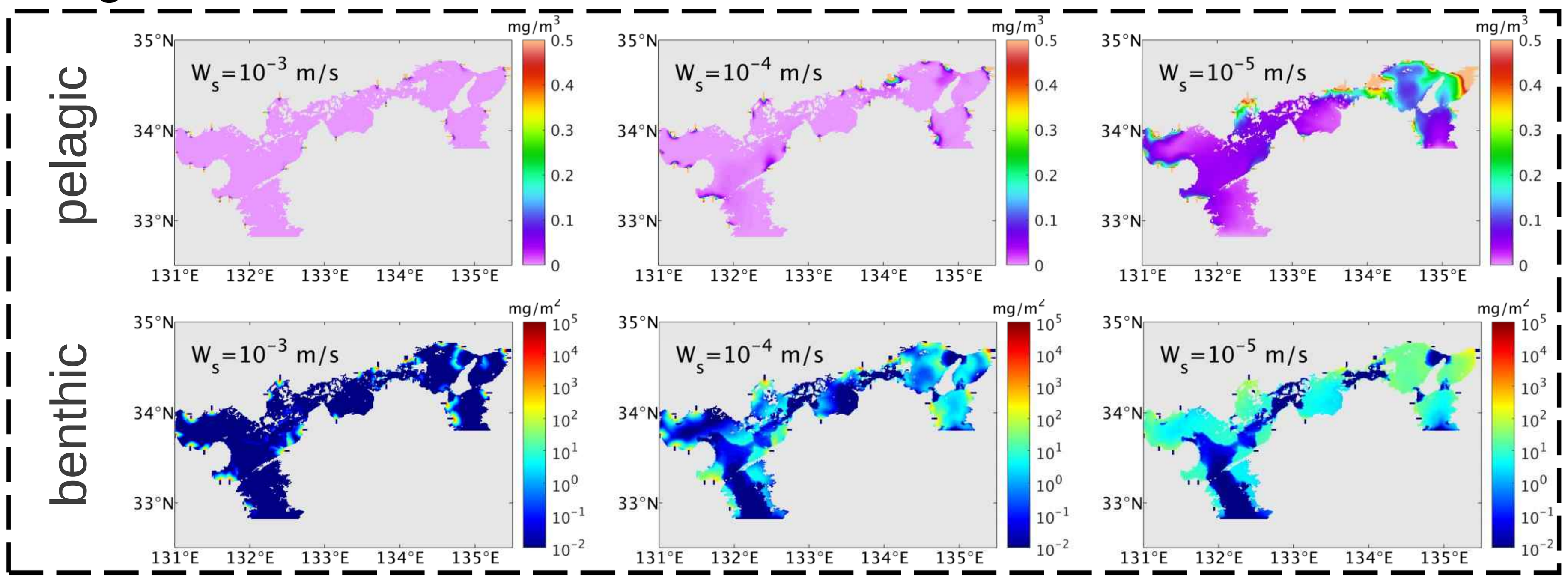
➢ **Bottom Boundary Condition:**

$$K_H \frac{\partial C}{\partial z} = E_k - D_k, z \to -H$$

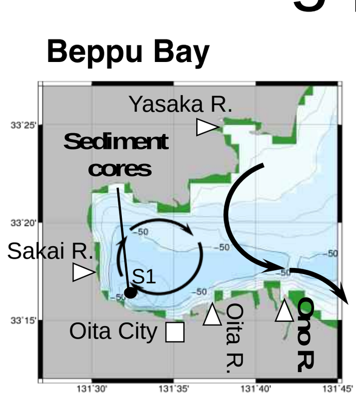
$$\begin{cases} E_k = E_0\left(\frac{|\tau_b|}{\tau_c} - 1\right), & \text{if } |\tau_b| > \tau_c \\ D_k = C_b w_s\left(\frac{|\tau_b|}{\tau_c} - 1\right), & \text{if } |\tau_b| < \tau_c \end{cases}$$

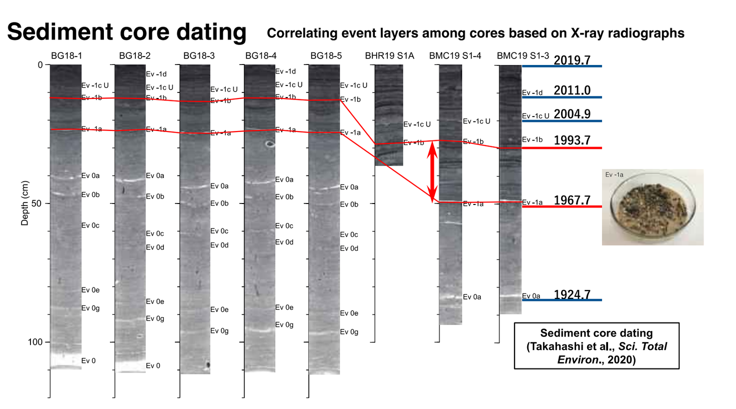
➢ **Degradation term:** $S = -\gamma C$

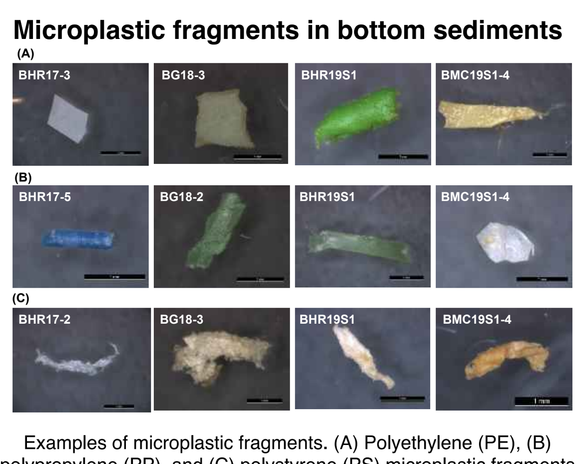




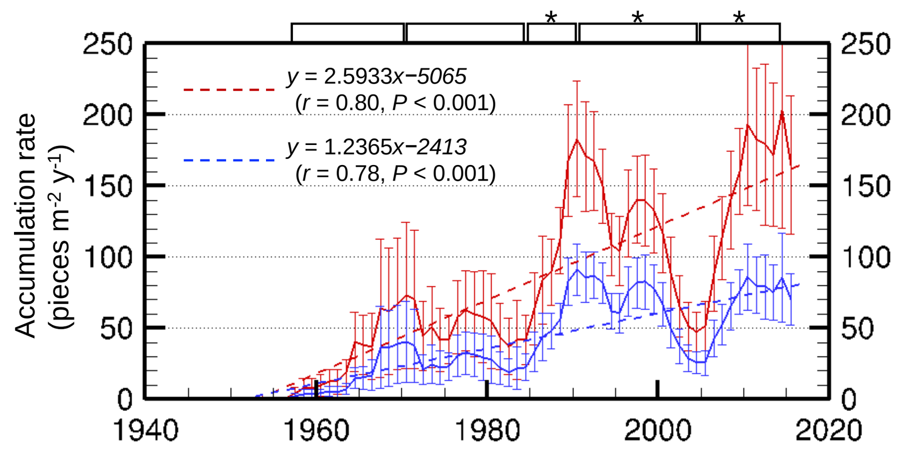
■ MP sinking process model

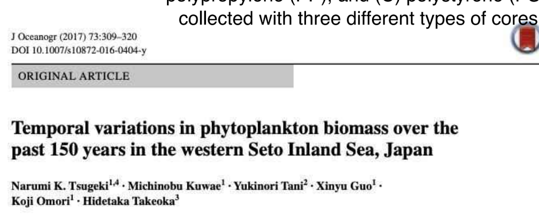






Examples of microplastic fragments. (A) Polyethylene (PE), (B) polypropylene (PP), and (C) polystyrene (PS) microplastic fragments collected with three different types of cores (BHR, BG, and BMC).

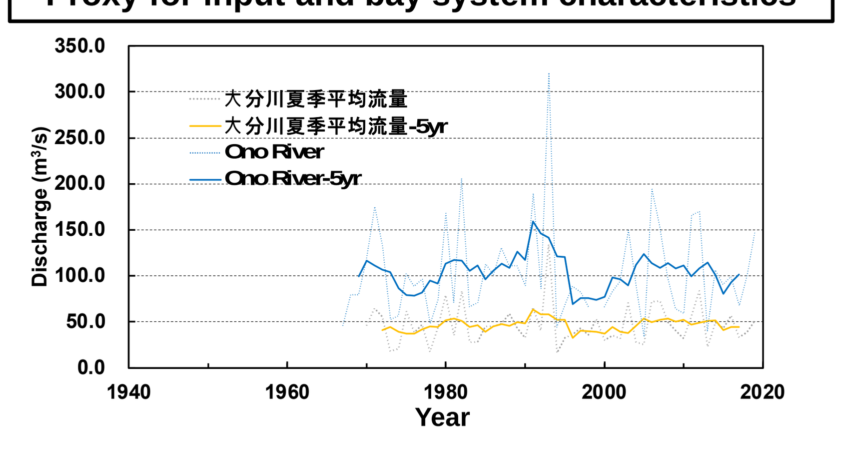


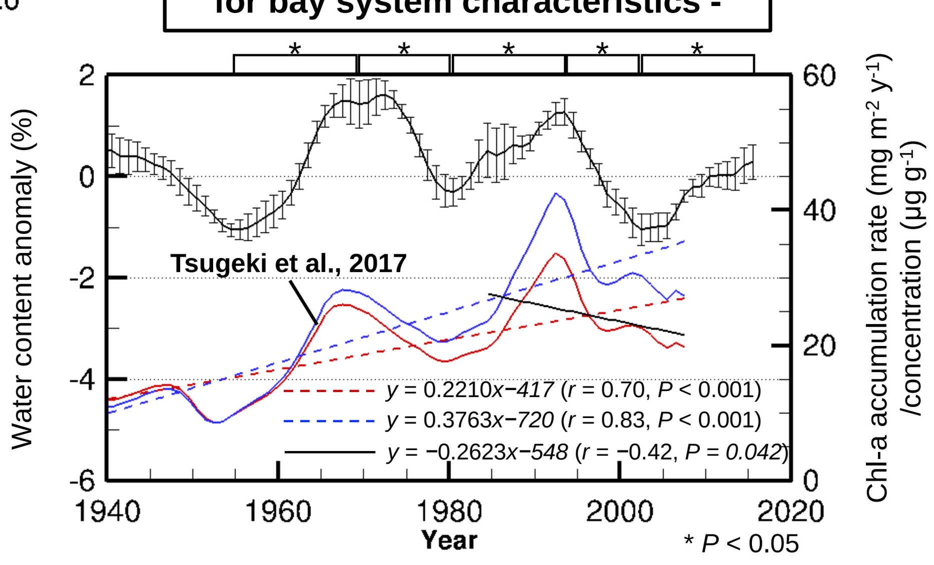


✓ Sampled 2009 at the deepest part
✓ Single core analysis (cell num. > 10¹ mL⁻¹)
✓ ^{210}Pb dating

Chl-a signals
- Biological activity-related proxy for bay system characteristics -

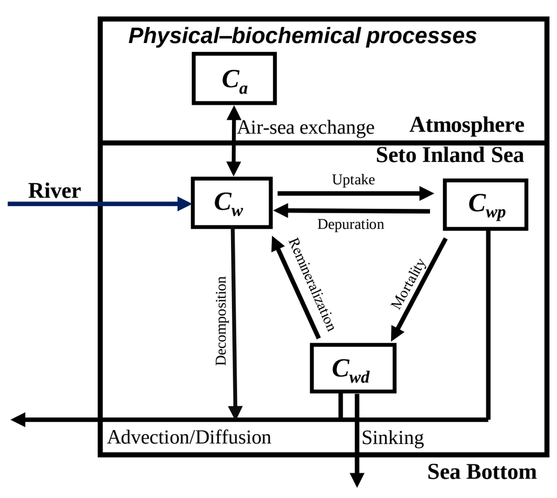
River discharge
- Proxy for input and bay system characteristics -

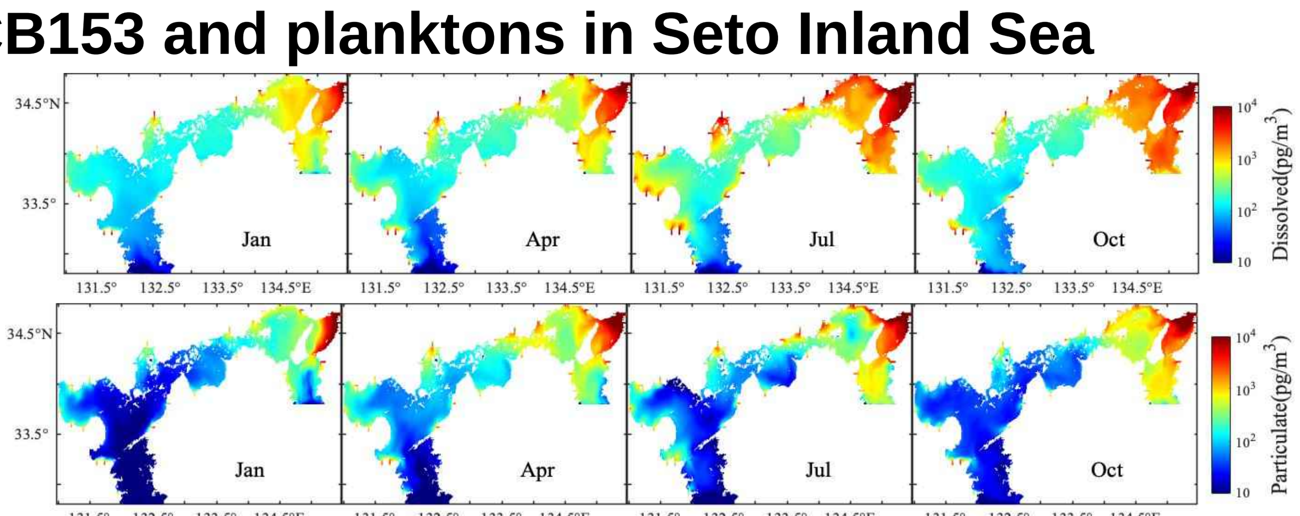




■ A 3D numerical model for the POPs and its interactions with planktons and polymers

➢ **Simulation of CB153 and planktons in Seto Inland Sea**



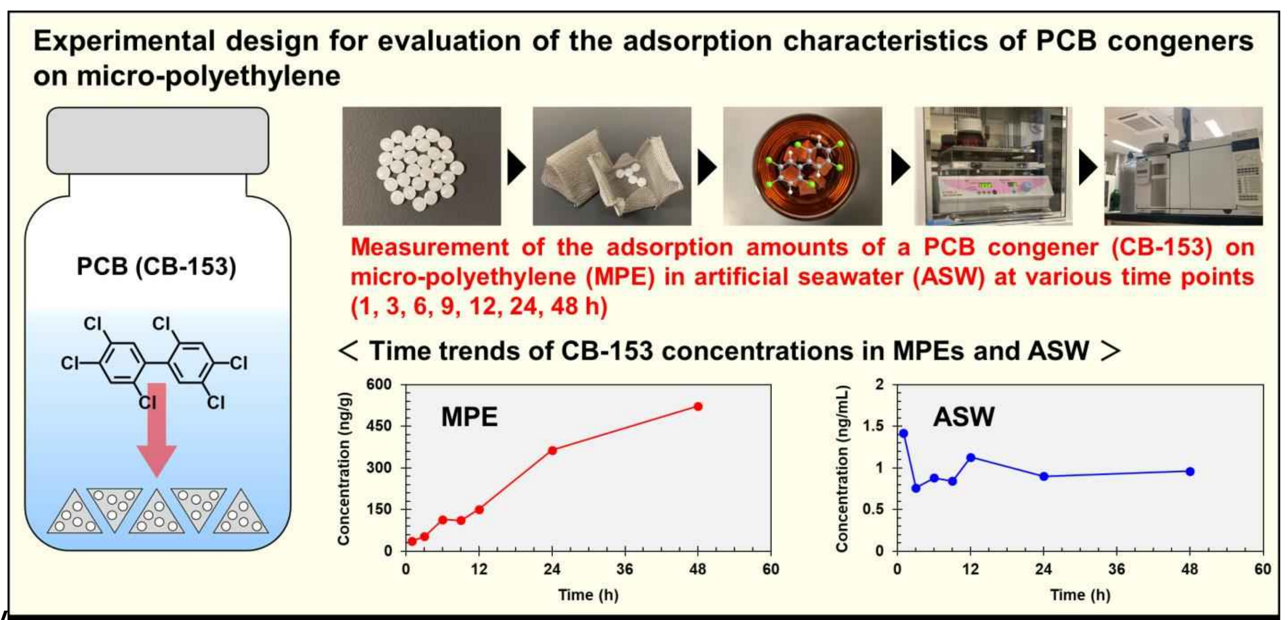


Distribution of dissolved and particulate concentration on sea surface

✓ Dissolved (C_w)
Phytoplankton surface CB153 concentration (C_{wps}),
Phytoplankton matrix CB153 concentration (C_{wpm}),
Detritus-bound (C_{wd})

✓ Dissolved > Particulate
Eastern part > Western part
High concentrations in estuary

Experimental design for evaluation of the adsorption characteristics of PCB congeners on micro-polyethylene



Our method enabled quantification and time-series evaluation of PCB adsorption on MPEs.
Adsorption experiments with various plastic types and PCB congeners will be conducted to collect data needed for environmental dynamic models.

No. A-11-17E

PJ: Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses

Theme : Development of accelerated evaluation of biodegradability in marine （E4）

Organization: Chemicals Evaluation and Research Institute, Japan

Contact: Takako KIKUCHI kikuchi-takako@ceri.jp

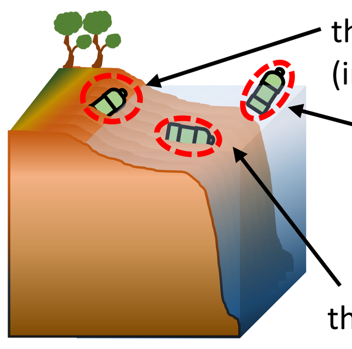
NEDO CERI MOONSHOT

1. Background and objective

In order to carry out a proper marine degradable plastic product design, it is important to know whether a plastic material is inherently biodegradable, degradation mechanism and safety etc. when exposed to marine inoculum.

◆International Standard about determination of aerobic biodegradation by ISO

(Each method is s simulation under laboratory conditions of each habitat found)



the habitat found in sandy tidal zone that, in marine science, is called eulittoral zone. (inoculum : sediment) [ISO 22404 : 2019 (by evolved CO_2)]

・offshore areas with low water currents and low tidal movements
・coastal areas with stronger water currents and tidal movements (inoculum : seawater) (ASTM D6691 is similar) [ISO 23977-1:2020(by evolved CO_2), ISO 23977-2:2020(by O_2 demand)]

the habitat found in different seawater/sediment-areas in the sea, e.g. in a benthic zone where sunlight reaches the ocean floor (photic zone) that, in marine science, is called sublittoral zone (inoculum : seawater/sediment interface) [ISO 19679:2016(by evolved CO_2)], ISO 18830:2016(by O_2 demand)]

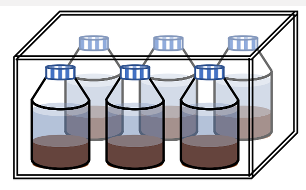
The evaluation of biodegradability in laboratory has some issues such as reproducibility and variability of the test, and long test period (6 months～24 months) .

◆ Issues in marine biodegradability evaluation

Field test

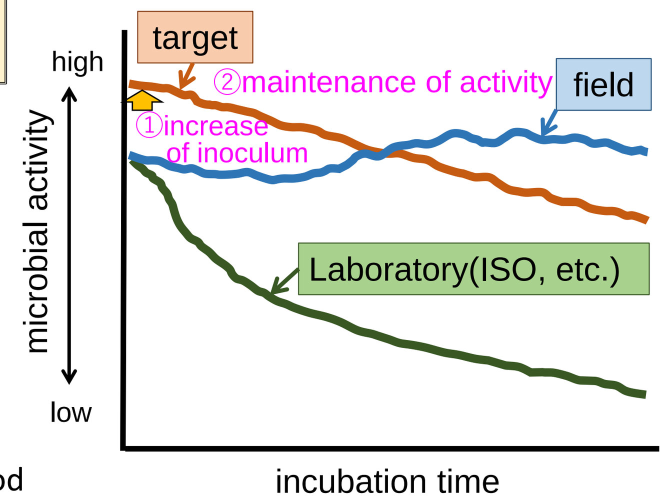
Investigate correlations between lab. tests and field

Laboratory test



Incubation is taking place in closed space for a long period

Diagram based on a model of enhancing microbial activity



In laboratory evaluations, the origin of the inoculum and the season of collection affect the results of the marine biodegradation.

The purpose of this study :
Development of accelerated evaluation of biodegradability in marine

2. Development of accelerated evaluation of biodegradability in marine

◆ Activation of initial inoculum by utilized microbial in sediment ◆

(1) Extracting of rich microbial in sediments to seawater

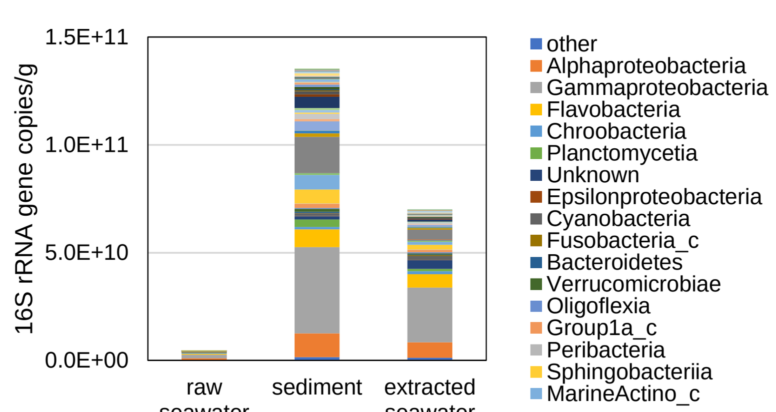
・The microbial density in sediment is generally higher compared to the density determined in seawater.

・ The use microbial in sediment is expected to diversification in inoculum.

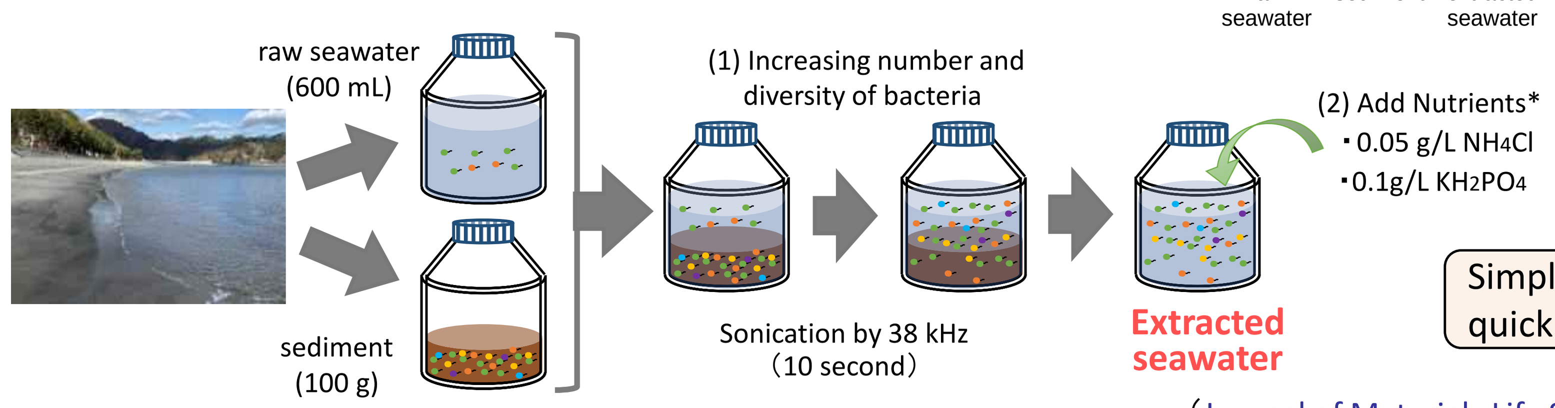
(2) Preservation of microbial activity by addition nutrients

・suppress microbial activity loss

Taxonomic classification of seawater and sediment at the phylum level of bacterial communities (corrected with 16S rRNA copies)



【 Preparation extracted seawater 】



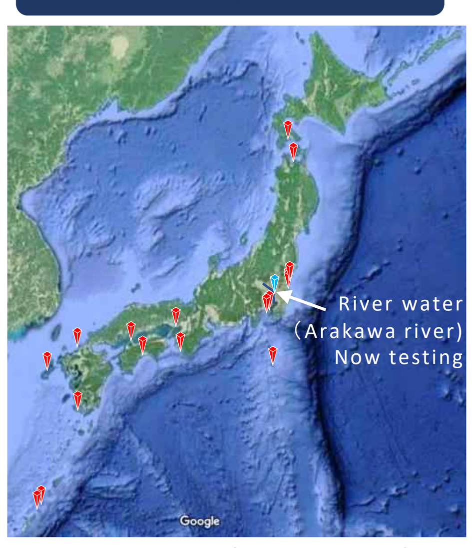
Simple operation and quick preparation

(Journal of Materials Life Society [33, 3 (2021)]

◆◆◆ Validation of development method ◆◆◆

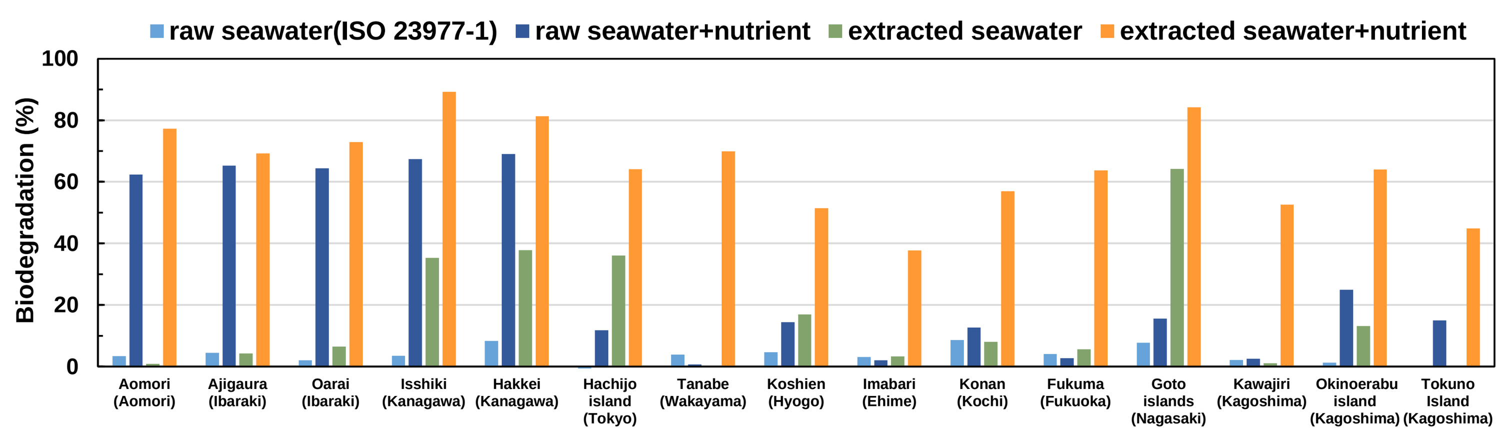
Marine biodegradability was measured using seawater and sediments at 15 sites in Japan(From Hokkaido to Kagoshima Pref.)

Sampling points

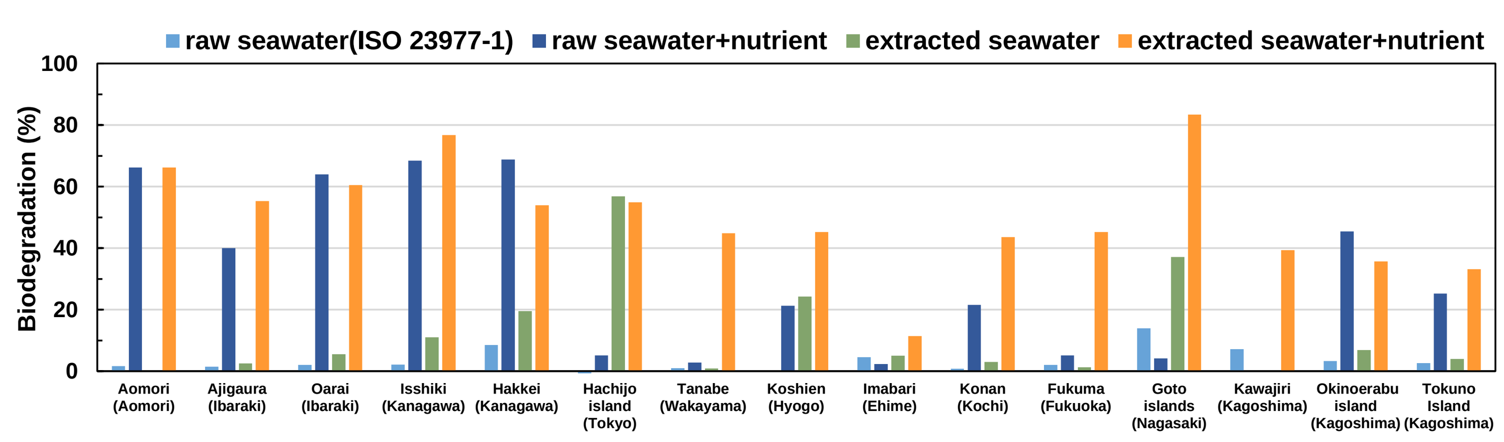


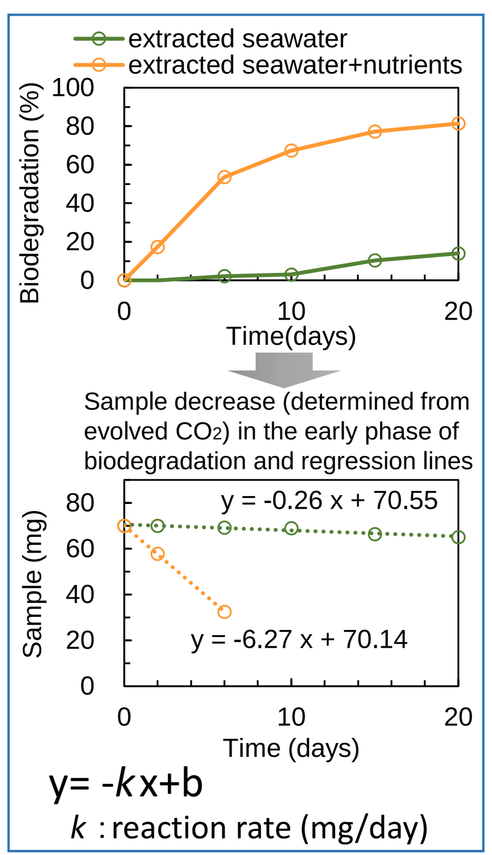
Period : 2021/11～2022/9

Biodegradation of cellulose after incubated for one month



Biodegradation of PCL after incubated for one month

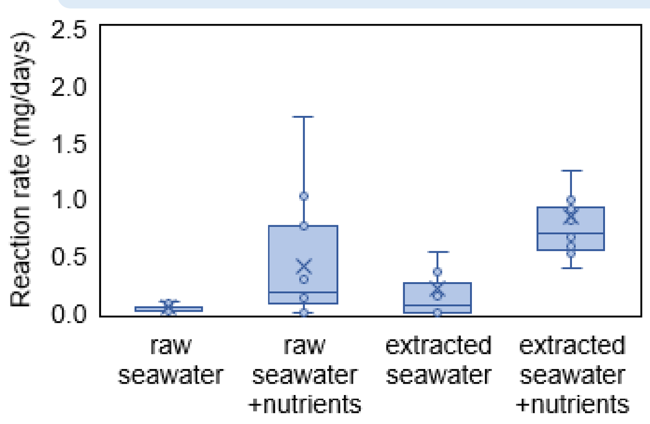




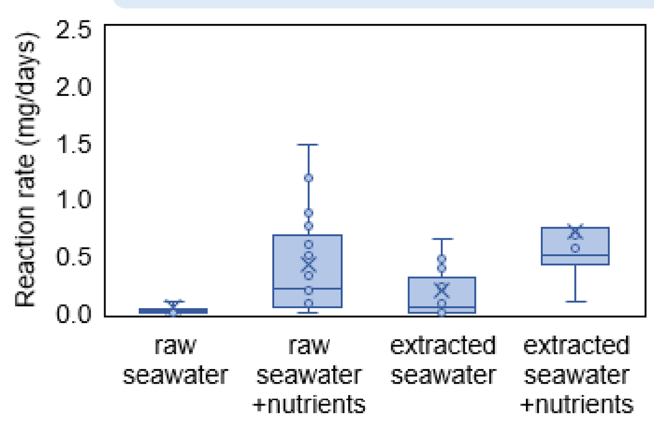
$y= -kx+b$

k : reaction rate (mg/day)

Reaction rate of cellulose



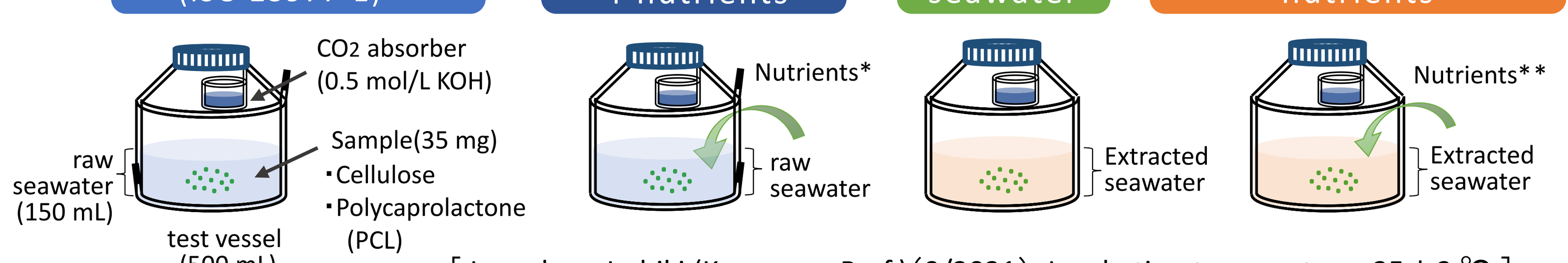
Reaction rate of PCL



At all locations, the development method could increase biodegradation rate and suppress the variations of test.

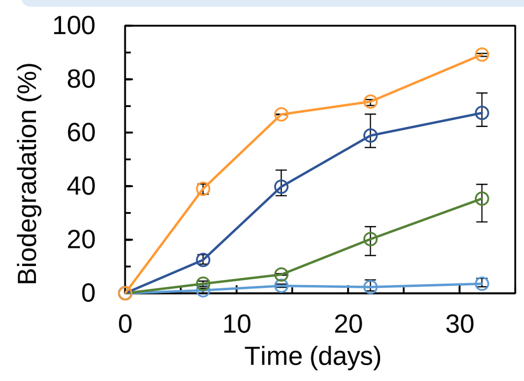
◆◆ Effectiveness of Development Methods ◆◆

raw seawater (ISO 23977-1)** | raw seawater ＋nutrients | extracted seawater | extracted seawater＋nutrients

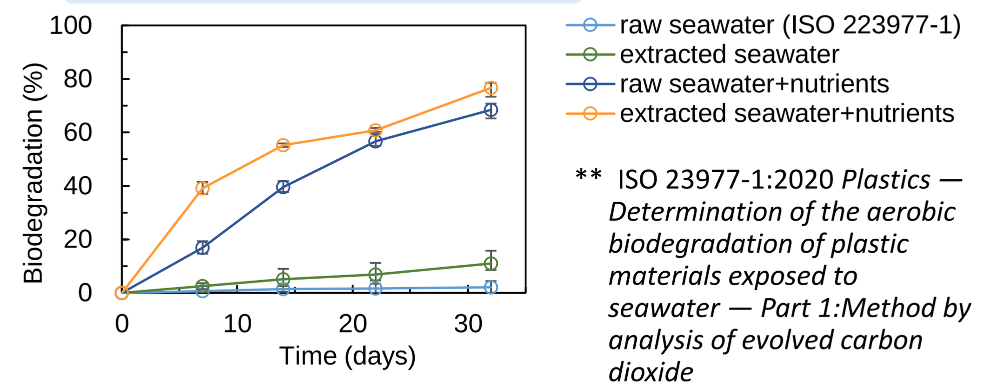


[Inoculum : Isshiki (Kanagawa Pref.)(9/2021), Incubation temperature : 25±2 ℃]

Biodegradation of cellulose

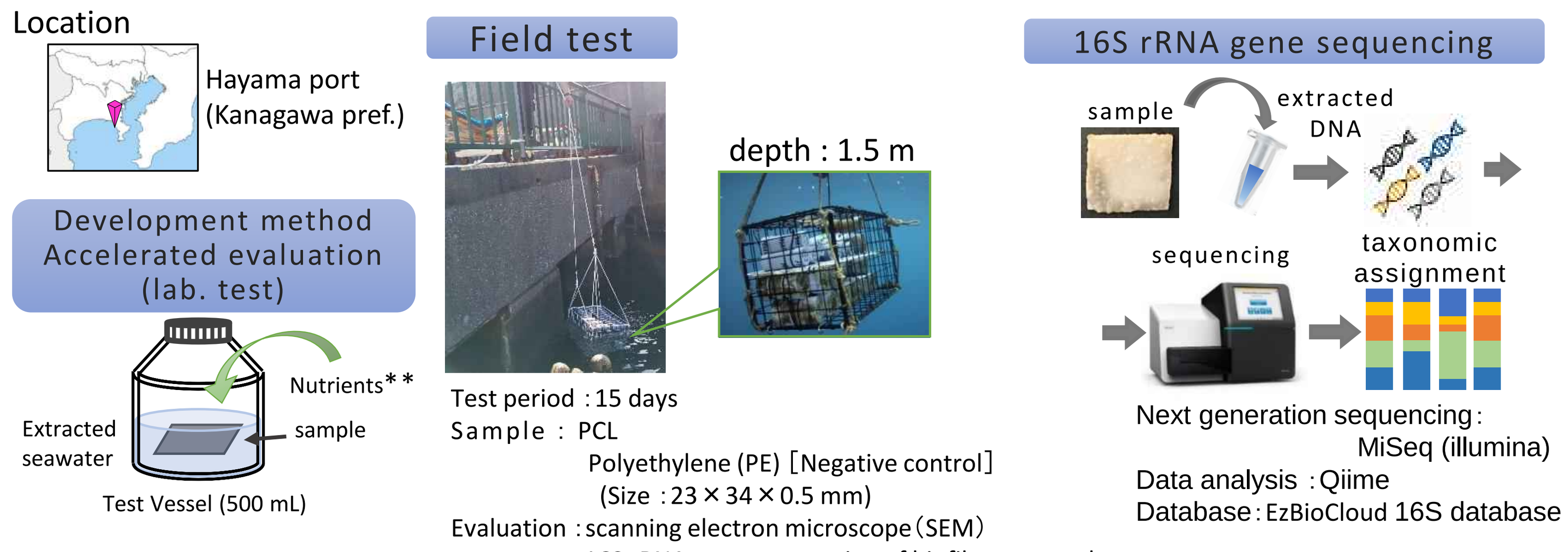


Biodegradation of PCL



** ISO 23977-1:2020 *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1:Method by analysis of evolved carbon dioxide*

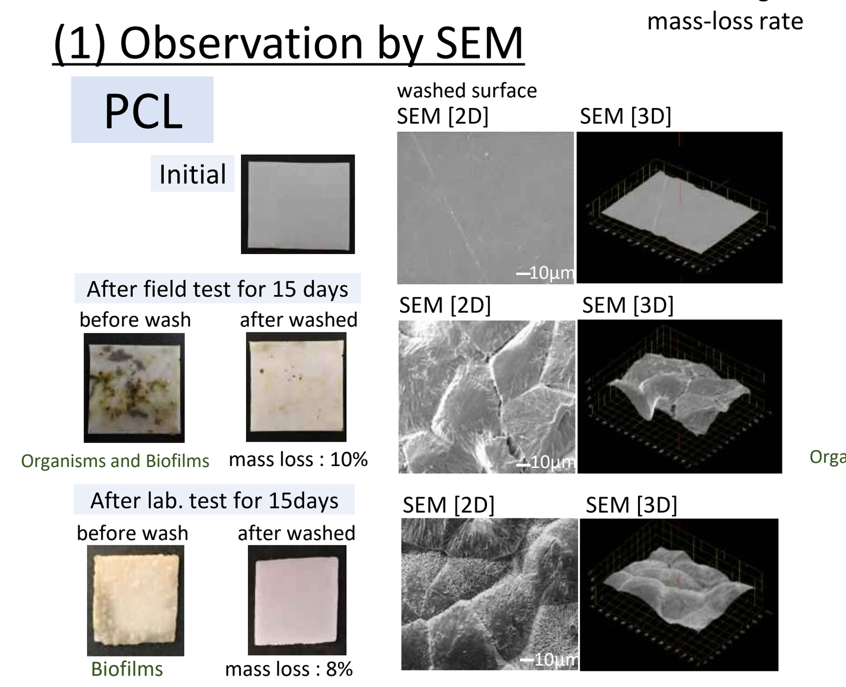
◆◆◆◆ Comparison of development method and field testing ◆◆◆◆

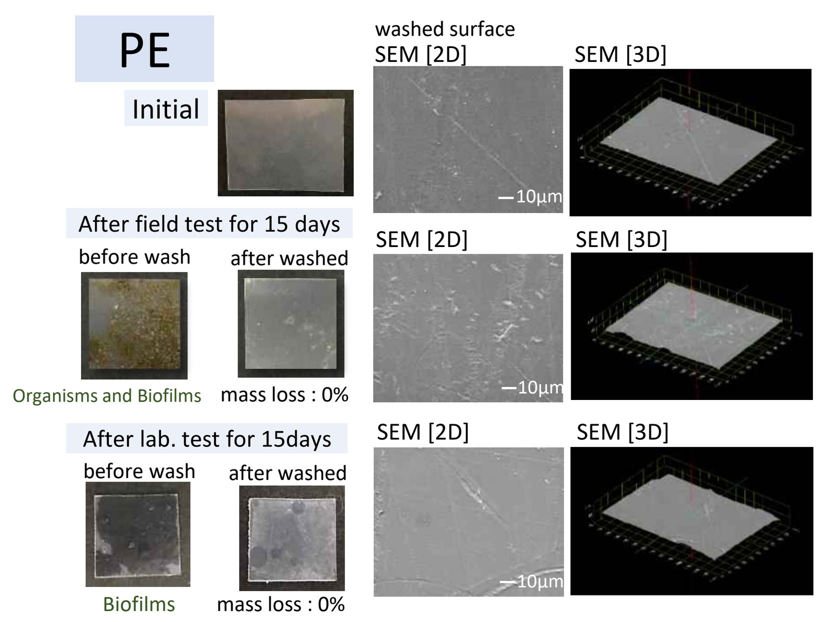


Test period : 15 days
Sample : PCL
Polyethylene (PE) [Negative control]
(Size : 23×34×0.5 mm)
Evaluation : scanning electron microscope (SEM)
16S rRNA gene sequencing of biofilm on samples
mass-loss rate

Next generation sequencing : MiSeq (illumina)
Data analysis : Qiime
Database : EzBioCloud 16S database

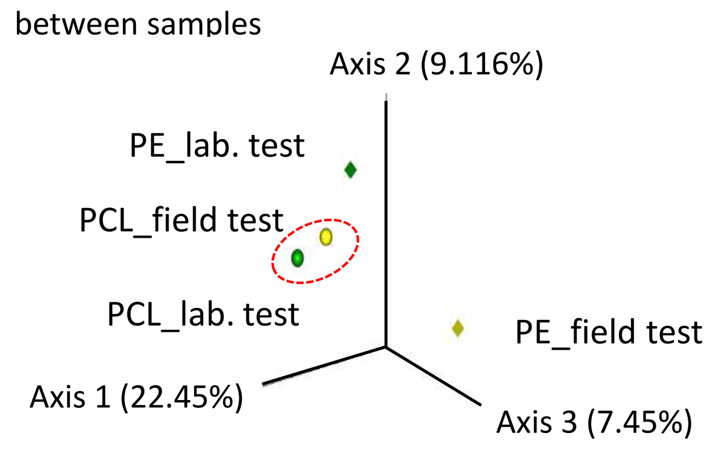
(1) Observation by SEM





(2) Taxonomic similarities between samples

Principal coordinate analysis of weighted unifrac distance metrics based on the taxonomic similarities between samples



In parallel with the marine biodegradability evaluation in the laboratory, the biodegradability in the field conditions was investigated.

The bacterial flora of the biofilm on PE in the laboratory was different from the one in the field.

The bacterial flora of the biofilm on PCL after the biodegradation test was similar between in the field and in the laboratory.

3. Conclusion

- We have developed accelerated evaluation method of biodegradability in seawater utilizing extracted microorganisms from marine sediments, furthermore the addition of nutrients accelerated the process.
- The bacterial flora of the biofilm on the biodegradable plastic after the biodegradation test was similar between in the field and in the laboratory. Therefore, it was confirmed that the laboratory evaluation could simulate the degradation in field conditions.
- In the future, by evaluating the relationship between biodegradation rate and amount of enzyme, we would like to quantify the acceleration ratio of the development methodology.